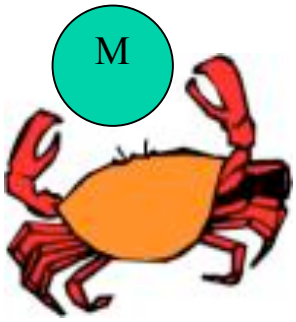
16th International Conference on Chelators (ICOC)

for the Treatment of Thalassaemia, Cancer and Other Diseases related to Metal and Free Radical Imbalance and Toxicity



25th Anniversary of DEFERIPRONE (L1)

Limassol, Cyprus
October 25-31, 2006.

WELCOME MESSAGE FROM THE PRESIDENT OF THE 16TH ICOC

Dear Colleagues and friends,

On behalf of the International Committee on Chelation, the local organising committee and the Postgraduate Research Institute, I would like to welcome you and to invide you to actively participate in the 16th International Conference on Chelators (ICOC) for the treatment of Thalassaemia, Cancer and other diseases related to metal imbalance and toxicity, which will be held in Limassol, Cyprus from the 27th to the 31st of October 2006.

Cyprus has an important link with metals and chelation therapy. It is possibly the only country named after a metal (copper). One in six native Cypriots is a heterozygote, asymptomatic carrier of thalassaemia and one in a thousand Cypriots is a thalassaemia patient. Partly as a result of iron chelation therapy, thalassaemia is no longer a fatal disease in Cyprus and many thalassaemia patients have children and belong to professional groups such as doctors, lawyers, academics etc.

The 16th ICOC, as in previous ICOC meetings, is expected to bring us up to date with the newest developments and provide a platform for future research in relation to metal and chelator chemistry, biochemistry, metabolism, pharmacology and toxicity, and the associated fields of science, environment, therapeutics and medicine.

Metal ions such as Fe, Cu and Zn and their metabolic pathways are essential for the growth and development of normal, neoplastic and microbial cells, whereas some metal ions are involved in carcinogenesis, inflammation and tissue damage. Moreover, toxic metals from environmental pollution such as Pb, Cd, Pu and U pose a serious threat to health, whereas other metals such as Gd, In and Tc are used in clinical diagnosis and others such as Pt and Al in therapeutics. Chelators and their metal complexes could be used to target and modify all such processes and conditions. They could also be used in combination with established therapeutics for improving the treatment of many diseases.

New and exciting developments have taken place over the last few years in the treatment of iron overload, the principal area where iron chelating drugs are currently used. The ICOC combination protocol of deferiprone (L1) / deferoxamine and other similar combination therapy protocols appear to be universally effective in bringing Thalassaemia, Myelodysplasia and other transfused patients in negative iron balance and in maintaining low, almost normal iron stores in the heart and other organs as detected by the MRI T2 and T2* techniques.

New iron chelating drugs have been developed, broadening the spectrum of therapy in thalassaemia and other iron loading conditions. Last year the new iron chelating drug Deferasirox (ICL670 or Exjade) was conditionally approval by the US FDA and also recently by the EU. Its clinical benefits are currently being investigated. Deferitrin, Triapine and Clioquinol have reached phase II clinical trials for the treatment of iron overload, cancer and Alzheimer¡¦s disease respectively. Several other chelators are also under clinical evaluation and many more are in the pre-clinical stage of development for metal detoxification.

Iron and other metal chelating drugs are generally classified as orphan drugs and are intended for orphan diseases. This partly reflects the unfortunate absence of progress or attempts to solve the problem of supplying iron chelating drugs to thalassaemia patients in developing countries, who are currently excluded from the benefits of chelation therapy as a result of high drug prices.

A number of interactive sessions have been planned in addition to those concerning the latest developments in the treatment of Thalassaemia and other iron loading conditions, Cancer research, Metal imbalance related diseases and Free Radical Toxicity related diseases. These comprise sessions on recent advances in the related areas of Haematology, Nutrition, Dermatology, Cardiology, Atherosclerosis, Medical diagnosis such as MRI, Environmental pollution and Drug design and development.

A meeting between patients and a panel of experts has been organised, as in previous ICOC conferences. Additionally, a meeting regarding the organisation of an International Society on Chelation and Metals (ISOCAM) and the venue of the 17th ICOC will also be held.

In the meantime arrangements have also been made to publish selected papers of this year's proceedings in the International Journal Hemoglobin.

Finally, a number of excursions and social events will be organised for the 16th ICOC to allow participants to relax following the usual vivid discussion and debate, which takes place during the scientific sessions. These events will familiarise newcomers with the unique scenery of Cyprus, Cypriot history and traditions and the warm hospitality of its people. A clean, blue sea and a sandy beach conveniently located just beside the hotel, also awaits, offering the possibility of a relaxing and tranquil swim.
I look forward to your participation and hope that you will enjoy your stay in Cyprus.

George J Kontoghiorghes
(Chairman of the ICOC Committee and President of the Conference)

WELCOME MESSAGE FROM THE HONORARY PRESIDENT OF THE 16TH ICOC

This year I hope to enjoy an ICOC meeting in Cyprus for the third time. This country is the perfect venue for such a conference for several reasons. First of all physicians and scientists from Cyprus have contributed considerably to the improvement of management of thalassaemia, including prevention and treatment of progressive and devastating iron overload. Active involvement of the well informed local and international patient organisations contributed to this outstanding performance. A second reason is that Cyprus is an attractive country with beautiful scenery, a rich history, an attractive climate all year round, a friendly population and excellent accommodations for having a conference. The 16thICOC should, therefore, provide a perfect opportunity for experienced and junior scientists to present recent progress in scientific and clinical investigation related to iron and other metal toxicity, thalassaemia, other iron overload diseases and iron chelators. I am confident that George J Kontoghiorghes and his co-workers will be able to organize once again a highly successful and pleasant ICOC meeting in Cyprus.

Jo Marx (Honorary President of the Conference)

WELCOME MESSAGE FROM THE VICE PRESIDENT OF THE 16TH ICOC

Welcome to Cyprus, the island of Aprodite,the godess of beauty and love. Ancient civilizations as old as 10000 years have been discovered in the island, where ancient castles, churches, mosaics and museums are visited by more than 2 million tourists every year. The natural beauty of the island with the sandy beaches, the forests in the mountains, the warm climate and the hospitality of the people of Cyprus are further attractions for the the tourists visiting the island.
Cyprus has a population of about 800000, 80% are Greek Cypriots and 18% Turkish Cypriots. Many overseas workers from all parts of the world are also living here and involved in the economic growth of the island. Cyprus has recently joined the European Union and because of its position in the southeast corner, close to the Middle East and North East Africa, is considered as one of the most sensitive border areas of the whole European Union. Unfortunately, one third of the population has been displaced from their homes since the Turkish invasion of 1974 and the north part of the island has since then been occupied by the Turkish army. Most people here in Cyprus are hopefull that the European membership will bring a resolution to the Cyprus problem and stability to the area, for the benefit of all the people of Cyprus and of future generations.

Annita Kolnagou (Vice President of the Conference)

Scientific and Organising Committees of the 16th ICOC

ICOC INTERNATIONAL COMMITTEE

G. J. Kontoghiorghes – Chairman (Limassol, Cyprus)
V. Eybl (Pilsen, Czech Republic)
J. J. M. Marx (Utrecht, The Netherlands)
A. Maggio (Palermo, Italy)
P. Töndury (Berne, Switzerland)
C. T. Peng (Taichung, Taiwan)
A. Kolnagou (Paphos, Cyprus)
I. B Afanasiev (Moscow, Russia)
D. R. Richardson (Sydney, Australia)

LOCAL ORGANIZING AND SCIENTIFIC COMMITTEE

President : G J Kontoghiorghes
Vice President : A Kolnagou

Members:

L. Antoniou	**A. Efstathiou**	**Ch Kontos**	**K Simamonian**
D. Diamantis	**E Eracleous**	**K.Kyriacou**	**G Violaris**
Ch Economides	**Ch Fessas**	**Y Michaelides**	**D Yazman**

INTERNATIONAL SCIENTIFIC COMMITTEE

Honorary President : JJM Marx (Utrecht, Netherlands)

President : G J Kontoghiorghes (Limassol, Cyprus)

Vice Presidents:
A Kolnagou (Paphos, Cyprus). A. Maggio (Palermo, Italy). C-T Peng (Taichung, Taiwan)

Secretaries:
D R Richardson (Sydney, Australia). P Tondury (Berne, Switzerland).
I B Afanasiev (Moscow, Russia)

Members:
A Aessopos (Athens, Greece)
A Aydinok (Izmir, Turkey)
L Cai (Luisville, USA)
C De Luca (Rome, Italy)
E. Eracleous (Nicosia, Cyprus)
S Fukuda (Chiba, Japan)
S Gotsis (Athens, Greece)
M Hopp (Tel Aviv, Israel)
K Kyriakou (Nicosia, Cyprus)
L Korkina (Moscow, Russia)
W Kruszewski (Warsaw, Poland)
AV Lebedev (Moscow, Russia)
G Liu (Utah, USA)
K Pantopoulos (Montreal, Canada)
P Ponka (Montreal, Canada)
MA Santos (Lisbon, Portugal)
E D Weinberg (Bloomington, USA)
MT Wilson (Colchester, UK)
JC Wood (Los Angeles, USA)

VENUE: MIRAMARE BEACH HOTEL, LIMASSOL, CYPRUS.

SCIENTIFIC SECRETARIAT AND LOCAL ORGANIZING COMMITTEE

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PROGRAMME SUMMARY

FIDAY 27TH OCTOBER

15.00-18.00 h Registration:

18.00 h Opening
Welcome Addresses by Local and International Organisations

18.30 h Kontoghiorghes GJ: Recent advances in natural sciences. Chelation in applied sciences and medicine.
18.50 h Marx JJM: Recent advances in medicine. From the iron age to new genes and new diseases
19.10 h Kolnagou A: Welcome to Cyprus

19.30 h Welcome Reception: Miramare Beach Hotel: Drinks and Snacks

SATURDAY 28TH OCTOBER

Session 1: 9.00- 10.30 h
Iron Metabolism, Imbalance and Toxicity

Chair: Marx JJM and Richardson DR
Ponka P: Recent Advances in physiology and pathophysiology of iron metabolism
Pantopoulos K: Iron regulatory proteins: role in health and disease
Afanasiev I B: The role and control of free radicals in medicine
Weinberg ED: Iron out-of-balance: a risk factor for acute and chronic disease
Round Table Discussion

10.30- 10.50 h Tea Break /Poster Viewing

Session 2: 10.50- 12.50 h
Clinical Studies: Effective Chelation Therapy using Deferiprone (L1) / Deferoxamine Combination in Thalassaemia patients

Chair: Aessopos A and Aydinok Y
Tondury P et al: Over 17 years of experience with deferiprone in Switzerland
Manz C et al: A randomized controlled trial comparing the combination therapy of deferıprone (L1) and desferrıoxamıne (DFO) versus L1 or DFO monotherapy ın patıents wıth thalassemıa major
Tsironi M et al: Effects of combined chelation therapy on serum ferritin levels and T2* values
Yazman D et al: Comparison of the efficacy of combined chelation of deferiprone and deferoxamine with other treatments on myocardial iron load in thalassaemia patients
Kolnagou A et al: Normalisation of serum ferritin, cardiac and liver iron in thalassaemia patients using the ICOC chelation protocol of deferiprone / deferoxamine combination
Round Table Discussion

12.50- 14.00 h Lunch Break

Session 3: 14.00- 15.30 h

Pathological Aspects of Cardiotoxicity and other forms of Iron Overload Toxicity. Cardioprotection by Deferiprone (L1)

Chair: Wood JC and Pharmaki K

Kyriacou K et al: Loss of myofibers and disruption of myocytes is the potential mechanism of iron overload toxicity in cardiomyopathy in iron loaded thalassaemia patients. Histopathological and electron microscopy findings.

Aydinok Y et al: Randomised controlled 1-year study of daily deferiprone plus twice weekly desferrioxamine compared with daily deferiprone monotherapy to assess NTBI and total iron excretion in patients with thalassaemia major

Spino M: Exploring the mechanisms underlying the cardioprotective effects of deferiprone

Peng CT: The cardiac function improvement of thalassemia patients in using deferiprone (L1)

Round Table Discussion

15.30-15.50 h Tea Break/Poster Viewing

Session 4: 15. 50 - 17.20 h

Comparative Studies of Iron Overload Toxicity in Various Organs and Different Categories of Iron Loaded patients. The Effects of Chelation Therapy.

Chair: Peng CT and Kolnagou A

Wood J C et al: Renal iron deposition occurs in chronically transfused sickle cell disease but not thalassemia major

Ramazzotti A et al: Myocardial iron overload and myocardial fibrosis in thalassemia intermedia versus thalassemia major patients: a comparative multicenter study

Farmaki K et al: Improvement of glucose metabolism in patients with thalassaemia major who undergo combined chelation therapy with deferoxamine and deferiprone

Vini D et al: Fertility in thalassaemia female patients: is there something new?

Round Table Discussion

OPEN-SESSION:17.30-19.00h

Patients meet the doctors: Questions and Answers session between an expert panel of doctors and patients / parents.

Panel of Experts:

(Aessopos A, Aydinok Y, Farmaki K, Kolnagou A, Maggio A, Marx JJM, Peng CT, Tondury P, Wood JC)

20.00 h Gala Dinner: Miramare Beach Hotel (Mermaid Restaurant)

SUNDAY 29th OCTOBER

Session 5: 9.00-10.30 h

Cardiomyopathy. Diagnostic Criteria and Chelation Therapy in Thalassaemia and Other iron Loading Diseases

Chair: Tondury P and Lu C

Aessopos A et al: Congestive heart failure and treatment in thalassemia major. Update on the effect of chelation therapy

Wood JS et al: Iron cardiomyopathy in a patient with sickle cell anemia.

Ramazzotti A et al: ECG changes in thalassemia patients: correlations with myocardial iron overload and myocardial fibrosis
Tsironi M et al: Predictive ECHO- DOPPLER indices of left ventricular impairment in β-thalassemic patients with ten years follow up
Round Table Discussion

10.30 – 10.50 h Tea Break /Poster Viewing

Session 6: 10.50 – 12.00 h
The Role of MRI T2 and T2* in Iron Overload Diagnosis and the progress of Chelation Therapy

Chair: Gotsis ED and Wood JC
Wood JC: The use of MRI for assessing iron overload and the progress of iron chelation therapy.
Gotsis ED: The uses and differences of MRI T2 and T2* in the determination of iron overload in iron loaded thalassaemia patients.
Kolnagou A et al: Maintenance of normal serum ferritin, cardiac and liver iron levels in a thalassaemia patient using deferiprone (L1) monotherapy for five years. A case report.
Round Table Discussion

Session 7:12.00 –13.00 h
Emergency Medicine and Experimental Chelators for Minimising Actinide Toxicity

Chair: Kruszewski M and Pantopoulos K
Fukuda S et al: Chelating agents for clinical application in the treatment of uranium and plutonium poisoning in radiation emergency medicine
Jin Y: DTPA administration protocol for radiological emergency medicine in nuclear fuel reprocessing plant
Fukuda S et al: Effects of CBMIDA administered orally and intraperitoneally on the removal of depleted uranium in rats
Round Table Discussion

13. 10 – 14.30 h Lunch Break
14.30 Excursion to Curium and Paphos. Dinner in Paphos.

MONDAY 30TH OCTOBER

Session 8: 9.00- 10.30 h
The Role of Iron Chelating Drugs in Cancer Prevention and Treatment

Chair: Ponka P and Weinberg ED
Richardson D R et al: Recent advances in the development of cancer therapeutics in relation to iron metabolism
Kyriacou K et al: Genetic epidemiology of breast cancer; results of a population based study in Cyprus
Simunek T et al: Assessment of pyridoxal isonicotinoyl hydrazone (PIH) and its analogs as cardioprotectants in anthracycline-induced cardiomyopathy.
Kontoghiorghes et al: New approach in the design and anticancer targeting activity of experimental and clinically used iron chelating drugs
Round Table Discusssion

10.30 – 10.50 h Tea Break / Poster Viewing

Session 9: 10.50 – 12.40 h
Iron Metabolism, Chelators and Antioxidants in Skin Pathology and Ageing

Chair : Wilson M T and Afanasiev IB
Korkina L: Iron in the skin physiology and pathology
De Luca C et al: Antioxidant/chelator-based individual treatments as a tool for the prevention and treatment of the aged skin
Cesario A et al: Nitrosylation of human glutathione transferase p1-1 with dinitrosyl diglutathionyl iron complex in vitro and in vivo.
Pourzand C et al: Caged-iron chelators as powerful prodrugs to protect the skin cells against iron-mediated lysosomal damage and necrotic cell death.
Lebedev AV et al: Iron chelators and free radical scavengers among naturally occurring hydroxylated 1,4-naphthoquinones
Round Table Discusssion

12.40 - 14.00 h Lunch Break

Session 10: 14.00 – 15.50 h
Metabolic Aspects of Nitric Oxide, Free Radicals and Iron in Health and Disease

Chair: Korkina L and De Luca C
Chaikovskayia N et al: Influence of NO-spin trap Fe-DETC on morphology of brain tissue, antioxidant activity and cerruloplasmin-transferrin system in blood serum in case of acute hypoxia-ischaemia using antioxidant mexidant and NOs-inhibitor iminobiotin.
Richardson DR et al: Nitrogen monoxide (NO)-mediated iron mobilization from cells is linked to NO-induced glutathione efflux via MRP1
Kruszewski M et al: Differential action of permeable and non-permeable iron chelators on formation of dinitrosyl iron complexes in vivo
Afanasiev IB: Nitric oxide and superoxide are mediators of chronic complications and premature aging in thalassemia and other hereditory diseases
Liu G et al: Skeletal free radicals mediated by iron: a new therapeutic target of postmenopausal osteoporosis using oral chelation
Round Table Discusssion

15.50-16.10 h Tea Break /Poster Viewing

Session 11:16.10 -18.00 h
Advances in the Design of New Treatments in Iron Metabolic Disorders

Chair: Maggio A and Kontoghiorghes GJ
Wilson MT: Hemoglobin based blood substitutes
Weinberg ED: Development of protein iron chelators as pharmaceutical agents
Richardson DR et al: Mitochondrial-permeable iron chelators prevent cardiac hypertrophy in the mouse model of Friedreich's Ataxia
Pantopoulos K et al: Iron-dependent degradation of apo-irp1 by the ubiquitin-proteasome pathway
Marx JJM et al: Do iron chelators influence progression of atherosclerosis?
Round Table Discussion

Session 12: 18.00 - 18.30 h:
Poster Discussion. Chair : Fuguda S and Yazman D

18.30 – 19.00 h: ICOC – ISOCAM MEMBERS MEETING

20.00 h: Dinner in a Taverna. Cypriot Cuisine, Live music and Dancing

TUESDAY 31ST OCTOBER

Session 13: 9.00 – 10.30 h
Design of New Chelators for the Treatment of Iron Metabolic Disorders and Free Radical Toxicity

Chair: Richardson DR and Pantopoulos K
Santos AM: New developments in trivalent cation chelators: extrafunctionalization and ligand-combined strategies.
Wilson MT et al: Comparative study of iron chelators as reducing agents for ferryl heme: A mechanism to protect against oxidative stress without iron chelation.
Srichairatanakool S et al: Investigation of 3-hydroxypyridin-4-one conjugate as a novel oral iron chelator.
Lebedev AV et al: Free radical conversion of catecholates associated with chelation of calcium and other group ii metal cations

10.30-10.50 h Tea Break

Session 14: 10.50 – 12.20 h
The Role of Copper, Zinc and Iron in Diabetic Cardiomyopathy

Chair: Santos MA and Weinberg ED
Lu C: Roles of zinc and copper in the diabetes and diabetic cardiomyopathy
Wenke F : Metallothionein regulation of hypoxia-inducible factor 1 in diabetic hearts
Lu C: Diabetic cardiomyopathy and metallothionein: what role of iron and copper play?
Li X et al: Cardiac protection of non-mitogenic human acidic fgf from oxidative damage *in vitro* and *in vivo*: potential application for the prevention of diabetic cardiomyopathy
Round Table Discussion

Session 15: 12.20 -13.00 h
Future Aspects of Chelation Therapy in Iron Overload and Other Diseases

Chair: Marx JJM
Maggio A et al: Lights and shadows in the chelation treatments
Kontoghiorghes GJ et al: Deferiprone (L1)-25th year anniversary. Its clinical use for the complete treatment of iron overload and possible uses in other diseases as monotherapy or in combination with deferoxamine, deferasirox , deferitrin and other drugs.

13.00 – 13.15 h Announcement of the 17th ICOC and Close.

POSTER PRESENTATIONS

1] Fukuda S et al: DEPLETED URANIUM REMOVAL EFFECTS OF CHELATING AGENTS IN COMBINATION WITH BICARBONATE IN RATS
2] Jang R-C et al: FROM COMBINATION THERAPY OF DESFERRIOXAMINE AND DEFERIPRONE TO ALONE USE OF DEFERIPRONE IN TRANSFUSION-DEPENDENT THALASSEMIC PATIENTS
3] Kolnagou A et al: RESULTS OF LONG TERM COMPARATIVE STUDIES IN THALASSAEMIA PATIENTS TREATED WITH DEFEROXAMINE OR COMBINATION THERAPY WITH DEFERIPRONE (L1). SUGGESTIONS FOR EFFECTIVE CHELATION PROTOCOLS.
4] Kontoghiorghes GJ et al: ESTIMATION OF CRITICAL THERAPEUTIC LEVELS OF DEFERIPRONE (L1) IN BLOOD OF THALASSAEMIA PATIENTS FOR MAXIMISING IRON REMOVAL AND MINIMISING IRON DEPOSITION AND TOXICITY IN THE HEART AND OTHER ORGANS
5] Pepe A et al: IN VIVO STANDARDIZED T2* MAP OF NORMAL HUMAN HEART, TO CORRECT T2* SEGMENTAL ARTEFACTS: A MULTISLICE, MULTIECHO T2* MRI APPROACH IN CARDIAC IRON OVERLOADED PATIENTS
6] Santos MA et al: HYDROXY (THIO)PYRIDINONES. NEW CHELATING AGENTS FOR POTENTIAL PHARMACOLOGICAL APPLICATIONS
7] Seixas JD et al: CARBON MONOXIDE RELEASING COMPOUNDS FOR THE TREATMENT OF INFLAMMATORY DISEASES
8] Theodosaki M et al: CHIMERIC STATUS OF CHILDREN WITH HAEMOGLOBINOPATHIES TREATED WITH STEM CELL TRANSPLANTATION
9] Tourkantoni N et al: IMMUNE AND NEURAL STATUS OF THALASSAEMIC PATIENTS RECEIVING DEFERIPRONE OR COMBINED DEFERIPRONE AND DESFERRIOXAMINE CHELATION TREATMENT
10] Zheng Y: EFFECT OF DIABETES ON THE CARDIAC PROTECTION INDUCED BY ISCHEMIA PRECONDITIONING

SPONSORS

The Organizing Committee and the ICOC committee thanks the following organisations and companies for their assistance, cooperation and financial support:

Postgraduate Research Institute of Science, Technology, Environment and Medicine(Limassol).
China Medical University (Taichung, Taiwan).
Neapolis University Planning Board, (Paphos).
United Kingdom Thalassaemia Society (London, UK)
Thalassaemia International Federation
Yermasoyia Municipality (Limassol)
Medical Diagnostic Centre Ayios Therissos (Nicosia)
Cyprus Airways
Cyprus Tourism Organisation (COT)
Cyprus Pharmaceutical Organisation Ltd
Limassol Cooperative Savings Bank Ltd
Coop. Kyperountas
Hellenic Bank
KEO Ltd
Booths:
Apotex (Canada).
Novartis Oncology (USA)

GENERAL INFORMATION

CONFERENCE VENUE

Miramare Beach Hotel
Limassol, 3721
Cyprus

Tel: +357 2588 8100 Fax: +357 2588 8200
E-mail: sales@miramare.com.cy
www.miramare.com.cy

CONFERENCE LANGUAGE

The conference language will be English. No simultaneous translation will be provided.

INFORMATION FOR SPEAKERS

ORAL PRESENTATIONS

All lectures will take place in the Lecture Hall of the conference room Artemis For speakers data (PC) projection, slide projection and overhead projection are available. The duration of Lectures is 15 mins and 5 mins are allowed for discussion. The round table discussion is 10mins maximum.

POSTER PRESENTATIONS AND EXHIBITION AREA

The poster and exhibition area is located in the conference room Athena on the opposite side of the corridor to the Lecture Hall, the conference room Artemis. Posters should be mounted on the boards by Saturday, October 28, 09:00 and then displayed till Monday, October 30, 18.30.
The space available for each poster is 0.75 m wide x 1.5 m high.

PUBLICATION OF THE 16TH ICOC PROCEEDINGS IN THE INTERNATIONAL JOURNAL HEMOGLOBIN

The authors of selected papers will receive an invitation to publish their papers (lectures or posters) in the Proceedings of the 16th ICOC by the guest editor of the proceedings and president of the conference G J Kontoghiorghes. Instructions to auhors for preparation of manuscripts will be the same as for those provided to the authors of articles in the journal Hemoglobin.

CONFERENCE REGISTRATION HOURS

During the Conference, the Secretariat and the Registration desk is located at the Conference venue, in the conference room Apollon on the opposite side of the corridor of the Lecture Hall, with the following time schedule:

Friday, October 27	15:00 – 18:00
Saturday, October 28	08.30 - 18.30
Sunday, October 29	09:00 – 13:30
Monday, October 30	09:00 – 18:30
Tuesday, October 31	09:00 – 12:00

REGISTRATION FEE

The registration fees for each category of participants have been displayed in the website www.icoc-isocam.org . As indicated only cash payments will be accepted for those paying at the registration desk

REGISTRATION FEE OF DELEGATES COVERS THE FOLLOWING

Welcome drink and scacks at the welcome reception (October 27). Cypriot Culture Night in the taverna Blue Lantern, which includes Dinner (October 30). Admission to the Scientific Sessions and Exhibition. Conference bag, materials and abstract book. A conference present. Refreshment during the breaks (tea, coffee etc). Certificate of attendance.

FOR ACCOMPANYING PERSONS

Welcome drink and scacks at the welcome reception (October 27). Cypriot Culture Night in the taverna Blue Lantern, which includes Dinner (October 30). A conference present.

CERTIFICATE OF ATTENDANCE

A certificate of attendance is available for all registered participants at the Registration desk.

LUNCHEONS AND REFRESHMENT
During the breaks, coffee/tea will be served in the Poster/Exhibition Area (opposite of the Lecture Hall). Luncheons are available in the conference participating hotels of the registered participants residing in them with the conditions stated in www.icoc-isocam.org.

TRANSPORTATION
There is a 24h taxi service, at taxi stations situated just outside Larnaca airport and the conference venue, Miramare Beach Hotel.

PARKING
The Hotel Miramare has its own parking facility. Parking is free of charge.

INSURANCE
The Organizing Committee will not accept any liability for personal injuries or loss or damage of property belonging to Conference participants and accompanying persons. Kindly check your personal insurance.

SOCIAL PROGRAMME

Friday, October 27, 2006 **Welcome Drink and snacks** **19.30 – 21:00**
Hotel Miramare (seafront area)

Informal Welcome Drink is held on Friday October 27 in the Conference venue (seafront area). Participants are invited to meet their colleagues.
Free for registered participants.

Saturday, October 28, 2006 **Gala Dinner** **20:00 – 13:30**
Miramare Beach Hotel (Mermaid restaurant)
Dinner with music
Tickets are available at the registration desk.

Sunday October 29, 2006 **Half day Excursion to Curion and Paphos.** **14.30 – 20:00**
Dinner in Paphos.
Tickets are available at the registration desk.

Monday October 30, 2006 **Cypriot Culture Night in the Taverna Blue Lantern** **20:00 – 24:00+**
Traditional, Cypriot food, Music and Dancing.
Free for registered participants and accompanying persons.

TOURS PROGRAMME

Pre and Post Conference Tours are available on request. For information please contact the registration desk.

16th International Conference on Chelation (ICOC)

ABSTRACTS OF ORAL PRESENTATIONS

The list is on an alphabetical order of the presenting authors

CONGESTIVE HEART FAILURE AND TREATMENT IN THALASSEMIA MAJOR UPDATE ON THE EFFECT OF CHELATION THERAPY

Aessopos, A and Kati M
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Pathogenesis Iron deposition in the heart is considered to play a central role in the development of congestive heart failure (CHF) in thalassaemia major (TM). Apart from cardiac iron overload, chronic anemia (Hb<8-10g/dL) with a subsequent high cardiac output state, arrhythmias, endocrinopathies (e.g. hypoparathyroidism), infections and genetic predisposition may also contribute.
Clinical picture Nowadays, in TM, heart failure usually occurs in the third or fourth decade of life and patients can be presented with symptoms of right- or left-sided heart failure, while biventricular involvement is the usual expression. CHF includes hepatomegaly, often accompanied by hepatic pain or abdominal discomfort on exertion, peripheral edema, neck vein distention, hepatojugular reflux, third heart sound gallop, arrhythmias as well as symptoms of increased pulmonary venous pressure such as nocturnal dyspnea, orthopnea, dyspnea on exertion.
Laboratory tests Hb levels, arterial blood gas as well as measurement of endocrine function (glucose, T3-T4, TSH, serum calcium) are necessitated. Chest X-ray, 12-lead ECG, Doppler echocardiographic and MRI study should also be done.
Treatment The patients with CHF should be hospitalized and closely monitored by everyday measurement of body weight and blood pressure and 24-hours urine secretion measurement. Everyday or second day measurement of electrolytes, urea and creatinine are also mandatory. Intensive chelation treatment with desferrioxamine (60-80mg/kg*/day i.v.) in combination with the oral chelator deferiprone (75-100mg/day) should be applied at once. Moreover, Hb levels should be kept around 10g/dL by regular blood transfusions. Diuretics, including loop diuretics and potassium-sparing agents, as well as ACE-inhibitors should be applied with respect to arterial blood pressure, daily diuresis, electrolytes and urea and creatinine measurements. If arrhythmias are present, intravenous administration of amiodarone is suggested. In case of renal output reduction or acute renal failure as a result of heart failure, treatment with intravenous positive inotropic agents should be considered for application. In the rare case of hypoparathyroidism, serum calcium levels should be corrected as CHF with coexistent low serum calcium concentration is extremely resistant to conventional treatment.

PREDICTIVE ECHO-DOPPLER INDICES OF LEFT VENTRICULAR IMPAIRMENT IN B-THALASSEMIC PATIENTS WITH TEN YEARS FOLLOW UP

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Background:. Early detection of cardiac function impairment by Echo Doppler indices can assist in modifying disease progression and prevent further cardiac damage, by applying intense chelation regimens.
Patients and Methods: We analyzed our database of Thalasssemia Major (TM) patients who had ten years constant therapy, consisting of blood transfusions every up to two weeks and desferoxamin 35mg/day, 4-5 days/week. All included patients should have initital (ten years ago) echo doppler study with normal Shortening Fraction (SF >= 0.3) and a recent examination within the last year. We identified all patients who developed impaired LV function in the last echo study and we attempted to find which of the indices that we measured in the early studies could predict the final impairment of LV function. To do this, we compared the initial study measurements with the corresponding ones of an equal number of age and sex matched controls, which we selected among the group of patients that did non develop LV systolic dysfunction. Subsequently we determined the sensitivity and specificity of the identified indices, by applying them to the entire study population.
Results: Of the 532 patients contained in our database, 315 patients were finally enrolled. Of them 12 developed LV systolic dysfunction (mean age 37.64±4.29 years). Of all echo measurements in the initial studies, only the LV end-systolic diameter index (2.11±0.07 vs 1.77±0.05, $p < 0.01$) and E/A ratio (2.20±0.26 vs 1.57±0.14, $p < 0.01$) differed significantly between patients and controls. Elevated LV end-systolic diameter index alone correctly predicts the development of LV systolic dysfunction in 8 patients, but incorrectly predicts its development in another 96 patients, who maintained normal LV function (sensitivity 67%, specificity 68%). E/A yields correct predictions in 6 patients, and incorrect in 80 (sensitivity 50%, specificity 74%). The two indices together yield correct predictions in 5 patients, and incorrect in 30 (sensitivity 42%, specificity 90%).
Conclusions: Increases in the end systolic index of the left ventricle and in the E/A ratio were the earliest pathological findings in those patients that finally developed LV systolic dysfunction. These parameters could thus be used as predictors of heart dysfunction, with relatively good sensitivity and specificity. This could help identify those individuals that are in need of intensive chelation and more thorough investigations.

THE ROLE AND CONTROL OF FREE RADICALS IN MEDICINE

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Experimental evidences of formation of reactive free radicals under physiological and pathophysiological conditions became one of critical developments in biology and medicine of 20th century. In accord with early findings of radiation biology free radicals formed in the reactions catalyzed by enzymes (xanthine oxidase, NADPH oxidase, etc.) or by the oxidation of low-molecular substrates were considered as harmful intermediates responsible for the initiation of numerous pathologies. However, the discovery of two "physiological" radicals superoxide and nitric oxide forming under strictly physiological conditions changed completely such a primitive point of view: it became clear that oxygen and nitrogen free radicals participate in important enzymatic processes (cell signaling, endothelium relaxation, phagocytosis, and many others) and therefore major task is not the simple suppression but regulation of free radical formation. This conclusion is correct for numerous pathologies associated with overproduction of reactive free radicals such as cardiovascular diseases, inflammation, or hereditary diseases (thalassemia, Fanconi anemia and other hemolytic anemias). Now we have many methods of regulation and control of the levels of free radicals in living organisms: the use of free radical scavengers and antioxidants, particularly nontoxic natural substrates and vitamins (vitamins C and E, glutathione, bioflavonoids), the mimics of antioxidant enzymes such as SOD and glutathione oxidase, the substrates of antioxidative enzymes (for example the substrate of NO synthase arginine) and so on. Another class of free radical inhibitors are chelators, which are able to suppress the formation of reactive hydroxyl radicals by removing of catalytically active iron from an organism or by the formation its inactive complexes.

In conclusion it should be stressed that the success or failure of antioxidant therapy depends on many factors including the correct and selective choice of inhibitors or promoters of free radical processes. (For example ascorbic acid and alpha-tocopherol are the efficient inhibitors of lipid peroxidation and protein oxidation while regulation of the superoxide and NO levels should be used SOD mimics or flavonoids). I am certain that the understanding of mechanisms of the overproduction and deregulation of free radicals in various pathologies will result in the successful application of antioxidative compounds and chelators for the treatment and prophylaxis of many pathologies.

NITRIC OXIDE AND SUPEROXIDE ARE MEDIATORS OF CHRONIC COMPLICATIONS AND PREMATURE AGING IN THALASSEMIA AND OTHER HEREDITORY DISEASES

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Major physiological radicals nitric oxide and superoxide are important mediators of many damaging processes in thalassemia and other hereditary hemolytic anemias. For example intravascular hemolysis, a common feature of thalassemia, is associated with a state of endothelial dysfunction characterized by reduced nitric oxide (NO) bioavailability, pro-oxidant and pro-inflammatory stress. It has been shown that a decrease in the NO levels is a characteristic feature of aging processes. At the same time the role of NO in thalassemia and sickle cell disease has been uncertain for some time. Thus it was earlier showed that nitric oxide production may increase in thalassemia. However the last studies demonstrate that intravascular hemolysis resulted in the reduction of NO bioavailability in thalassemia and sickle cell disease. This phenomenon becomes especially serious for aged patients. We earlier proposed [1] that deregulation of the superoxide/NO balance can be a cause of mitochondrial aging and pathological disorders. Therefore in addition to intravascular hemolysis a decrease in NO bioavailability can be caused by the enhancement of superoxide production due to iron-stimulated oxidative stress through the formation of peroxynitrite and the suppression of NO-depended inhibition of mitochondrial cytochrome c oxidase.
Thus the stimulation of nitric oxide production and the inhibition of superoxide overproduction could be useful addition to traditional chelation therapy of thalassemic patients. It should be stressed that traditional antioxidants vitamins E and C are ineffective scavengers of both nitric oxide and superoxide. Therefore it is quite possible that arginine therapy (the substrate of NO synthase) [2] or the application of bioflavonoid rutin (superoxide scavenger) [3] could be recommended for the treatment of thalassemic patients in addition to chelation therapy.

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RANDOMISED CONTROLLED 1-YEAR STUDY OF DAILY DEFERIPRONE (DFP) PLUS TWICE WEEKLY DESFERRIOXAMINE (DFO) COMPARED WITH DAILY DEFERIPRONE MONOTHERAPY TO ASSESS NTBI AND TOTAL IRON EXCRETION IN PATIENTS WITH THALASSAEMIA MAJOR

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Introduction: Non-transferrin bound iron (NTBI) is potentially toxic and may play a catalytic role in the initiation of free radical reactions. Eliminating toxic iron by decreasing tissue iron to safe concentrations is the major goal of iron chelation therapy. The effects of oral DFP or the combination of oral DFP and s.c. DFO, on NTBI levels and total iron excretion have not been compared previously in a randomised controlled study. Patients treated with standard DFO were also included in the comparison without randomisation.

Methods: A total of 24 patients with thalassaemia major were randomised into one of the following 2 arms; DFP (LIPOMED AG, Switzerland) was given at a daily dose of 75 mg/kg either in combination with DFO (NOVARTIS, Switzerland) (40-50 mg/kg twice weekly) or as single agent. Five patients registered in the DFO control arm received 40-50 mg/kg s.c. DFO 5 days a week. All patients had been treated with DFO prior to the study and had a wash-out phase without any iron chelating medication of 2 weeks before initiation of the trial. Plasma NTBI was measured by HPLC at baseline and at weeks 1, 12, 26 and 54 taken 12h after the last dose of DFP or 24h after the last DFO dose standardised to blood transfusion interval. Liver iron concentration (LIC) was measured in biopsies prior to study start and after 1 year of treatment. Total body iron excretion (TBIE)/day was calculated from the following formula = (iron transfused/year (mg) + (LIC at T_o - LIC at T_{1y}) x 10.6 x body wt in kg) /number of days treatment between biopsies.

Results: The results show that under the conditions of this trial, a trend towards increase in NTBI was observed in all treatment groups during the first 3 months of study compared to baseline levels. However, a stabilisation in NTBI was observed in patients receiving the combination therapy or DFO monotherapy whereas single agent therapy with DFP was associated with a significant increase in NTBI ($p=0.002$) after 1 year. The average decrease of LIC was most pronounced in patients receiving the combination therapy (-32%). Furthermore, the addition of s.c. DFO twice weekly to daily DFP therapy resulted in higher TBIE ($p=0.059$) and significantly higher ratio of iron excretion to iron intake ($p=0.037$) in comparison to daily DFP monotherapy and was the regimen associated with the highest and most efficient iron excretion. There was also a higher rate of iron excretion and iron balance achieved by the combination therapy in comparison to standard DFO therapy ($p=0.06$).

Discussion and conclusion: This study demonstrates that the combination regimen is superior to DFP monotherapy in eliminating toxic iron more efficiently by reducing tissue iron and inducing a negative iron balance.

ROLES OF ZINC AND COPPER IN THE DIABETES AND DIABETIC CARDIOMYOPATHY

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Both zinc and copper are essential minerals that are required for various cellular functions. Therefore, excess amount zinc and copper contribute to toxic effects. In contrast, deficiency of these minerals also involve in the initiation and promotion of certain diseases. Diabetes is a metabolic syndrome with either insulin defect or insulin resistance. Zinc plays a critical role in insulin conformation and secretion, and also has an insulin-like function. Therefore, zinc deficiency was found to be associated with the increased incidence of type 1 diabetes and with the decreased tissue's response to insulin in type 2 diabetes. In addition, zinc deficiency was also associated with the increased inflammation and arteriosclerosis due to the increased ratio of zinc to copper, which is considered as a risk of cardiovascular diseases. Supplementation of copper chelator has demonstrated the preventive effect of diabetic cardiomyopathy in human and animal studies, suggesting the possible toxic effect of the copper. The copper toxic effect may be either due to an increased absorption of copper or a decreased secretion of zinc, leading to a decreased ratio of zinc/copper. Correspondingly, supplementation of zinc to diabetic animals and patients provided significant benefits for several pathogeneses, including diabetic cardiomyopathy. Therefore, this presentation will summarize our own and other studies to discuss the roles of zinc and copper in the diabetes and diabetic cardiomyopathy (Supported, in part, by research grants from ADA, Philip Morris USA, Inc. and JDRF).

DIABETIC CARDIOMYOPATHY AND METALLOTHIONEIN: WHAT ROLE OF IRON AND COPPER PLAY?

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Cardiovascular disease is the most common cause of death in the diabetic patients and cardiomyopathy as one of these cardiovascular diseases in the diabetic patients can occur without vascular diseases. Diabetic cardiomyopathy is directly attributed to oxidative stress, therefore, antioxidant therapy has been attractive approach, but the outcomes of several clinical trails using various available antioxidants remain unsatisfied. New antioxidants or mimics are being currently developed. Using animal models, we recently demonstrated that metallothionein (MT) provided a significant prevention of diabetic cardiomyopathy through suppression of oxidative/nitrosative stress. Since MT is an endogenous, multiple-organ-existed and wide-range free radical scavenger and also highly inducible, potentially clinical application of MT is very attractive. To support this notion, we recently showed that supplementation of zinc to diabetic mice significantly prevented the development of diabetic cardiomyopathy along with a significant induction of cardiac MT, suggesting the feasibility for pharmaceutical approach to up-regulating endogenous cardiac MT to protect the heart from diabetes-induced damage.
This presentation will thus summarize our results on the prevention by MT of diabetic cardiomyopathy with an emphasis of its experimental evidence, possible mechanisms and clinical implication. In addition, possible role of iron overload in the pathogenesis of diabetic cardiomyopathy and its removal by MT will be also discussed

NITROSYLATION OF HUMAN GLUTATHIONE TRANSFERASE P1-1 WITH DINITROSYL DIGLUTATHIONYL IRON COMPLEX IN VITRO AND IN VIVO.

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Introduction: It has been recently shown by a previous work (1) that dinitrosyl diglutathionyl iron complex (DNDGIC), a possible *in vivo* nitric oxide (NO) donor, binds with extraordinary affinity to one of the active sites of human glutathione transferase P1-1 (GST) and triggers negative cooperativity in the neighboring subunit of the dimer. This strong interaction suggest a mechanism by which GST P1-1 may act as intracellular NO carriers or scavenger.
Results and Methods: We have reported the crystallographic structure of GST P1-1 with the DNDGIC bound in the active site, which, together with site-directed mutagenesis studies, could demonstrate the crucial role of the catalytic residue Tyr 7 acting as a ligand for the iron complex in the active site. It has been solved at high resolution in collaboration with Professor Michael Parker. These data provide direct support that GSTs can be nitrosylated *in vitro*. Electronic Paramagnetic Resonance (EPR) and kinetic studies on *E. coli* cells overexpressing GST P1-1, upon exposure to 2 mM nitrosoglutathione (GSNO), suggest that human GST P1-1 can also be nitrosylated *in vivo* inside the cell (2).
As a part of this study we have tried to evaluate if these bacterial cells underwent possible alterations of their proteome caused by GSNO exposure. So we have analyzed the soluble fraction of the bacterial cells, obtained at different times upon GSNO treatment, using a 2-dimensional electrophoresis approach.
Discussion and Conclusions: The crystal structure provides the first structural view of the DNDGIC complex and first view of it bound to a protein and therefore may be of value for other studies concerning these biological complexes.Experiments with *E. coli* cells, overexpressing human GST P1-1, demonstrated for the first time the *in vivo* formation of the DNDGIC complex, upon exposure to GSNO. The presence of the overexpressing GST P1-1 in bacteria is crucial to stabilize this complex, in fact free DNDGIC has a very short half life in solution and is never observed in the cells (2). Also, the partial inactivation of GST P1-1 observed in *E.coli* extracts can be explained on the basis of previous *in vitro* experiments of GST P1-1 inhibition (1) and present structural findings. It has been reported previously that nitric oxide (administered to the cells as nitrosothiol) can cross the cell membrane and be regenerated as nitrosothiol inside the cell (3). This appears to have occurred in our experiments, where upon exposure to exogenous GSNO we observed a rapid formation of DNDGIC complex inside the cell. The importance of thiol (mostly GSH) in favoring this interaction between NO and Fe has been pointed out (or as reductant to remove iron from proteins or as a ligand for iron coordination along with NO) (4) and as result there is always formation of DNDGIC complexes. Our studies add a significant piece of knowledge to this mechanism: this complex, with a very short life in solution, once formed may be trapped and stabilized by GST P1-1.The proteomic study has revealed by mass spectrometry about twenty proteins differentially regulated by GSNO. The preliminary findings suggest that NO exposure could affect some proteins involved in cellular growth in a relative short time, while, after a longer incubation, proteins involved in an antioxidative stress response could be activated

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INFLUENCE OF NO-SPIN TRAP FE-DETC ON MORPHOLOGY OF BRAIN TISSUE, ANTIOXIDANT ACTIVITY AND CERRULOPLASMIN-TRANSFERRIN SYSTEM IN BLOOD SERUM IN CASE OF ACUTE HYPOXIA-ISCHIMIA USING ANTIOXIDANT MEXIDANT AND NOS-INHIBITOR IMINOBIOTIN.

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Introduction: Diethyldithiocarbamate(DETC) complex with Fe(II) is a spin-trap widely using for NO detection in tissues. Acute hypoxia-ischimia is a very important problem in neurology, neonatology, traumatology and many other fields of medicine.
Methods: White rats (av.250gr\each)were used for the experiment (N=64). In the experiment model of acute hypoxia-ischimia included ligation of one carotid artery and exposition of animals in 8 % oxygen atmosphere for 60 minutes (Peeters-Scholte et al., 2002). Animals were divided into following groups: 1-control(n=8), 2- control+Fe-DETC(n=8), 3- hypoxia (n=8), 4-hypoxia+Fe-DETC(n=8), 5-hypoxia+Mexidant(n=8), 6-hypoxia+Mexidant+Fe-DETC(n=8), 7-hypoxia+iminobiotin(n=8), 8-hypoxia+iminobiotin+Fe-DETC (n=8). Estimation of brain damage was hold by morphometry (counting of oedema elements in the observing field) after fixation in 5 mkm slices. Summary antioxidant activity (SAA) and level of lipid hydroperoxides (LH) were measured by hemiluminescence. Parameters of cerruloplasmin-transferrin (Cp-Tr) system were measured by EPR-spectroscopy.
Results: It was shown that in case of Fe-DETC injection in control group morphometric parameters became much worse as 2,83±0,19 in 2 group comparatively to 0,71±0,17 in 1 group ($p<0,001$). Another effect was noticed when Fe-DETC was injected in condition of acute hypoxia-ischimia where morphometric parameters became better as 10,79±1,19 in 3 group comparatively to 4,10±1,36 in 4 group ($p<0,001$). In case of Mexidant injection as 6,42±0,71 in 5 group comparatively to 2,67±0,45 in 6 group ($p<0,001$), the same effect but even stronger developed in case of iminobiotin injection as 2,25±0,29 in 7 group comparatively to 1,24±0,32 in 8 group ($p<0,001$). At the same time it was shown in the experiments that in case of acute hypoxia-ischimia SAA decreases from 5,63±0,89 in the 1 group to -6,25±4,29 in 3 group ($p<0,001$). But in case of Fe-DETC injection parameters of Tr was unmeasurable
(too high) especially in case of acute hypoxia-ischimia and iminobiotin injection.
Discussion and conclusion: Thus, we can conclude that using of Mexidant and iminobiotin decreases neuronal damage in brain tissue in case of acute hypoxia-ischimia and injection of NO-spin trap Fe-DETC increases this positive effect and behaves like antioxidant, also influence SAA, increasing its level together with decreasing LH. Fe-DETC injection in case of acute hypoxia-ischimia also influence level of Tr in blood serum making it very high.

ANTIOXIDANT/CHELATOR-BASED INDIVIDUAL TREATMENTS AS A TOOL FOR THE PREVENTION AND TREATMENT OF THE AGED SKIN

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Iron is involved in skin ageing, and in many dermatological disorders, including hyper-, hypo-pigmentation, inflammation, porphyrias, infections and cancers. Possible mechanisms include simple deposition processes, activation/inactivation of enzymatic systems, impairment of phagocyte functions, interference with apoptosis. Dermatologist extensively advise antioxidant (AO) supplementation to prevent the skin tissue damage induced by excess exogenous free radical (FR) formation, occurring with ageing. Here we provide experimental data in vivo, on the rat model and on patients, performed by ESR, chemiluminescence, fluorescence, immunochemistry, spectrophotometry, HPLC techniques, to prove that , paradoxically, all technologies of skin rejuvenation, physical, chemical and surgery interventions, affect by different mechanisms the sensitive physiological FR/AO balance in the skin, with consequent consistent risks of skin and systemic adverse effects. In different experimental models of skin peeling, burning, excision wound, or laser treatment at the energies used for skin rejuvenation, performed on the rat shaved abdomen, analyses of skin biopsies and blood parameters demonstrated the occurrence of a severe topical and systemic oxidative damage. Data obtained from the blood of patients undergoing dermocosmetic surgical interventions (face peeling, blepharoplasty) demonstrated that, depending on dosage and duration of the application, dermo-cosmetic/surgical treatments can either induce or inhibit cutaneous free radical processes. In the animal models, we also demonstrated that the oral administration, pre- and post-treatment, of selected AO (RRR-alpha-tocopherol, coenzyme Q_{10}) and chelators (phenylpropanoids), associated with enhancers of aspecific immune defense (soy phospholipids, L-methionine), were significantly beneficial to skin wound healing and trophism. Based on these experimental data, it can be stated that aesthetic procedures induce a state of acute oxidative stress both locally in the skin and systemically. Combined oral/topical treatments must include AO/chelators combinations, inhibiting excess oxidative damage, and mild pro-oxidants, accelerating healing and skin cell growth

IMPROVEMENT OF GLUCOSE METABOLISM IN PATIENTS WITH THALASSAEMIA MAJOR WHO UNDERGO COMBINED CHELATION THERAPY WITH DEFEROXAMINE AND DEFERIPRONE

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Aim: To study the effect of combined chelation therapy in Glucose Metabolism in Thalassaemic patients (TMp).
Patients: 49 transfusion-dependent TMp (26 males, 23 females), aged 12–46 years, switched from Deferoxamine to combined chelation of oral Ferriprox® (25-30mg/kg t.i.d) and Desferal® (20-50mg/kg, 8-12h SC or IV 2-6days/week), in a 3-5 year regimen based on individual needs.

- 7 Thalassaemic patients TMp (14%) with Insulin Dependant Diabetes were excluded from the study
- Classification of remaining 42 TMp according to their glucose metabolism status was performed according to the WHO criteria. The normal group was further divided in two subgroups according to ADA.

Methods: Annual glucose tolerance tests oral (OGTT) and intravenous (IVGTT) were performed. Area under curve (AUC) was calculated for both glucose and insulin. We used SCHOMA in order to assess beta-cell secretion and ISI HOMA for Insulin sensitivity.

- Mean annual Ferritin was estimated according to monthly determinations by MEIA
- Non-invasive hepatic iron quantification was performed by annual Signa-MRI 1.5 Tesla, multi-echo T_2 & T_2^* sequences (CVi, General Electric Signa, Milwaukee, WI, USA).

Results: Mean Ferritin levels decreased from 2991± 2093 μg/L to 638±1345 (p<0.001)

1) Liver iron deposition also dramatically reduced over 3-5 years time after combined chelation MRI T_2L increased from 22,39±5,12 msec to 34,35±6,9 msec (p<0.001)
2) Following combined chelation and the addition of Ferriprox®, Glucose Metabolism was improved.
   - This is proved by the reduction of Glucose-AUC in OGTT and the statistical significant differences in mean Fasting Blood Glucose & Glucose after 2 hours in OGTT.
   - The evidence of Insulin production is demonstrated by the increase of Insulin-AUC in OGTT & IVGTT and proved by the increment of SCHOMA model.
   - Insulin Resistance was reduced, as deduced by the reduction of Glucose-AUC in OGTT. Also there was an overall increment of insulin sensitivity ISI_{HOMA} with Combined chelation in most of TMp.

Conclusions: Combined chelation with Ferriprox® & Desferal® seems to be the treatment of choice for a significant decrease in body iron load as assessed by serum Ferritin levels and liver iron concentration by T_2L MRI. Not only abnormal glucose tolerance was reversed in a significant proportion of our patients, but the cumulative glucose response was also significantly improved with this regimen. Both insulin secretion and sensitivity were also noticeably increased. This fact indicates that the impairment of beta-cell function due to iron-induced toxicity may be reversible in some patients. More studies are necessary to better explain the most beneficial factors to Glucose metabolism during combined chelation.

CHELATING AGENTS FOR CLINICAL APPLICATION IN THE TREATMENT OF URANIUM AND PLUTONIUM POISONING IN RADIATION EMERGENCY MEDICINE

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Radiation workers are always exposed to a risk of contamination by radioactive materials such as plutonium and uranium in a radiation accident. So far, our chelation study of actinides focuses on the ability to reduce the risk of radiation-induced cancer by long-term internal exposure to plutonium. Recent reports have indicated that uranium induces various disorders such as disturbances in the reproductive and nervous system, fetal malformation, and dysfunctions of kidney and bone, probably due to the chemical toxicity.
Chelation therapy is an optimal method for reducing radionuclide-induced risks. Many chelating agents have been examined to identify as drug having the ability to accelerate excretion of radionuclides from the body with no side effects. DTPA is well known as a chelating agent that can remove plutonium from the body that has gained approval as a medicine in a few countries; the problem remains, however, that the ability to completely eliminate the risk of plutonium may be limited by accidental conditions. Therefore, new chelating agents that are more effective than DTPA are required. At present, it is of great importance to obtain chelating agents for uranium removal that can be used in humans. Chelating agents, such as CBMIDA, 3,4,3-LIHOPO, EHBP, deferiprone (L1) and 4,6-dimethyl-1-hydroxypyrimidin-2(1H)-one, have been examined. Judging by the data on the toxicity, many kinds of drugs will likely be necessary to prevent the various disorders described above, not only to accelerate excretion of uranium but also to cure the various types of damage induced by the so-called secondary effects. The present strategies for obtaining the drugs that can be used in persons contaminated by uranium and plutonium are to find out new chelating agents and apply drugs that approved as a medicine for other diseases. At any rate, the final goal is to establish a clinical approach using chelating agents that can release the risk and anxiety of radionuclide contamination.

EFFECTS OF CBMIDA ADMINISTERED ORALLY AND INTRAPERITONEALLY ON THE REMOVAL OF DEPLETED URANIUM IN RATS

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We examined the effects of Catechol-3,6-bis(methyleiminodiacetic acid) (CBMIDA) on the removal of depleted uranium (DU) in animals. Experiment I was carried out to examine the effects of CBMIDA in rats to which DU was intramuscularly injected. Male rats injected intramuscularly with 8 mg/kg DU were divided into seven groups. Rats in three groups were orally administered 240, 720 or 1200 mg/kg CBMIDA, and rats in three other groups were injected intraperitoneally with 240, 480, or 720 mg/kg CBMID for 3 days. Each administration began 30 min after the DU injection. One group acted as a control (no CBMIDA treatment). Experiment II examined the effects of CBMIDA against intraperitoneally injected DU. After the injection of 8mg/kg DU, CBMIDA was administered orally to rats in three groups, and was injected intraperitoneally into rats in three other groups. Each group received the same doses on the same schedules as described in Experiment 1. Rats were kept in a metabolic cage so that urine and feces could be collected for the measurement of DU concentrations, and they were killed 6 days later. The liver, kidney, femur and muscles were obtained for the measurement of DU concentrations, and serum was obtained for biochemical examinations.
Results. In Experiment I, significant increases in DU excretion and significant decreases in the DU concentrations in the organs were induced in all the CBMIDA groups in comparison with the no-CBMIDA treatment group by both the oral and intraperitoneal administrations. In Experiment II, the DU excretions increased in a dose-dependent manner, and the DU concentrations in the organs decreased significantly in the oral 720 and 1200 mg/kg CBMIDA groups and in the intraperitoneal 720 mg/kg CBMIDA group.
The results indicated that both oral and intraperitoneal administrations of CBMIDA were effective in reducing DU in the intramuscularly injected DU rats at doses over 240 mg/kg, and also at high doses in the intraperitoneally injected DU rats.

THE USES AND DIFFERENCES OF MRI T2 AND T2* IN THE DETERMINATION OF IRON OVERLOAD IN IRON LOADED THALASSAEMIA PATIENTS

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Introduction: Quantitative MRI (measurement of T2* and T2 relaxation times) have been proven to be sensitive probes in assessing iron overload in the liver and myocardium. This author suggests that combining the higher sensitivity of T2* with the higher specificity of T2 leads to a much more complete probe in determining not only total concentrations of iron but also the storage conditions of it (mainly ferritin and hemosiderin).

Methods: For cardiac T2* measurements the Brompton protocol was used, with a single breath-hold multi-echo gradient echo sequence. The same sequence was sufficient for liver T2* down to T2* ≈ 2 msec. Below that a different sequence was used with much shorter TE values (0.96 – 1.5 msec in steps of 0.1 msec). For T2 three different spin echo sequences were evaluated with special phantoms as well as patients: a single Hahn echo sequence repeated at various TE values (the Tim St. Pierre protocol)[1], a 4-echo multi-echo sequence and a Carr-Purcell-Meiboom-Gill (CPMG) multi-echo sequence.

Results: A plot of R2* (=1/T2*) vs. R2 (=1/T2) for the liver as well as the myocardium is not linear in the entire range of R2 and R2* values. This finding will be discussed.

Discussion and conclusion: Intrinsically T2* is a more sensitive probe for iron detection, and it deserves to be the backbone of any protocol. On the other hand, T2 has as higher specification for it can only detect effects due to chemical exchange, a phenomenon that requires soluble iron carriers like ferritin. Thus using both T2* and T2 one has the simultaneous advantages of high sensitivity and high specificity, and the relative concentrations of ferritin and hemosiderin can thus be estimated. Whether hemosiderin is more “dangerous” than ferritin or not remains to be proven, the presence of hemosiderin however coincides with iron levels that can lead to liver fibrosis or heart failure, thus being able to deduce its presence may be of value in iron overload assessment.

DTPA ADMINISTRATION PROTOCOL FOR RADIOLOGICAL EMERGENCY MEDICINE IN NUCLEAR FUEL REPROCESSING PLANT

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Introduction: Diethylene-triamine-pentaacetate (DTPA) is a calcium or zinc salt that is specifically used for treating people who have been internally contaminated with plutonium, americium and curium. Now first commercial nuclear reprocessing plant in Japan is in the testing operation and the importance of DTPA has been reevaluated. The DTPA administration protocol for contaminated workers was discussed.

Routes of incorporation: Inhalation, wound contamination and absorption via burn surfaces are the main routes through which nuclides are incorporated into the body. DTPA therapy will be initiated immediately to the workers who have significant incorporation of plutonium-238, -239, -240, -241, americium-241 and curium-244.

Protocols of DTPA administration: DTPA administration methods include inhalation, intravenous administration and application to wound or burn surface. As inhalation is the easiest among these administration methods, it enables administration at an early stage. The sets including a portable nebulizer and DTPA solution have been readied. Wound and burn surfaces should be washed with the normal saline solution to which DTPA has been added. In the case of individuals for whom the use of DTPA is known in advance to be contraindicated, such as individuals with nephrotic syndrome, Zn-DTPA is administered from the beginning. Zn-DTPA is also used for long-term administration, and the duration of administration is determined by monitoring nuclide excretion levels in the urine.

Conclusion: Necessity of chelating agents for use in radiation emergency medicine has been expanded again. The DTPA administration protocol in the nuclear fuel reprocessing plant was described.

NORMALISATION OF SERUM FERRITIN, CARDIAC AND LIVER IRON IN THALASSAEMIA PATIENTS USING THE ICOC CHELATION PROTOCOL OF DEFERIPRONE (L1) / DEFEROXAMINE COMBINATION

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Introduction: Many chelation protocols have been suggested for the effective treatment of iron overload over the last few years using monotherapies or combination therapies. The ICOC combination protocol of the administration of daily deferiprone (L1) (85-110 mg/kg) and of sc deferoxamine (DF) (40-60 mg/kg), at least 3 nights per week using a pump or the whole 24 h using an infusor, has been shown to be effective in reducing serum ferritin levels and cardiac iron stores.
Objective: To examine the efficacy of the International Committee on Chelation (ICOC) protocol for the clearance of excess iron from the heart and the liver of thalassaemia patients and the maintenance of low iron stores, by monitoring MRI T2* and T2, as well as serum ferritin levels.
Patients and Methods: We have evaluated the ICOC combination therapy protocol in 8 thalassaemia patients (5 males, 3 females) who have been showing signs of low compliance with DF. The patients had variable serum ferritin levels (0.56 - 3.8 mg/l) and mostly heavy cardiac iron (4.7 -24 ms) and liver iron (0.8-4.4 ms) levels, as detected by MRI T2*. The monitoring period of the combination therapy ranged from 19 to 54 months.
Results: The ICOC combination L1/DF therapy was well tolerated by all the patients and there were no serious toxic side effects. There was a substantial reduction in serum ferritin levels in all the patients, in some cases reaching and maintaining normal levels (0.05- 0.6 mg/l). Similarly, there was a substantial reduction of cardiac iron as shown from an increase of cardiac MRI T2* (20-44 ms), reaching normal levels in all the patients. In 2 patients excess cardiac iron was cleared within 10 months. Excess liver iron was also reduced in all the patients over the same period reaching normal values (MRI T2* > 6.3 ms) in most patients as assessed by MRI T2* (3.5-35 ms). Similar findings were observed using the MRI T2 assessment method.
Discussion and Conclusion: The ICOC combination protocol of L1/DF appears to be effective in the rapid clearance of excess iron from the heart and the liver as detected by MRI T2* and T2. In 2 patients the rate of clearance of iron from the heart was observed within 10 months, which is faster than that previously reported in patients using high doses of either L1 or DF monotherapy. Liver iron levels were also reduced in all the patients but at a slower rate than cardiac iron, presumably because of the much higher capacity of the liver for storing iron than the heart. No toxic side effects were reported during the study. It appears that the ICOC L1/DF combination protocol is highly effective for the rapid clearance of excess iron from both the heart and the liver.

1] Kontoghiorghes GJ, Kolnagou A. Lancet 2003; 361:184.2] Peng CT et al Eur J Haematol 2003: 70: 392-7. 3] Kolnagou A, et al Brit J Haematol, 2004; 127:360-1.

MAINTENANCE OF NORMAL SERUM FERRITIN, CARDIAC AND LIVER IRON LEVELS IN A THALASSAEMIA PATIENT USING DEFERIPRONE (L1) MONOTHERAPY FOR FIVE YEARS. A CASE REPORT.

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Introduction: Effective treatment of iron overload over the last few years has been achieved using combination therapy protocols of deferiprone (L1) and deferoxamine (DF), such as the ICOC combination protocol. However, in some cases the administration of either drug may not be applicable due to toxic side effects, low compliance, low efficacy or high cost. We report here a case of a female thalassaemia patient with iron overload cardiomyopathy, where iv deferoxamine was not sufficiently effective, but the administration of L1 caused a clearance of excess cardiac iron, a reversal of the cardiomyopathy and maintainance of normal serum ferritin, cardiac and liver iron levels.
Patient and Methods: A 45 years old female thalassaemia patient was admitted with a diastolic heart failure 9 years ago despite that she was regularly using subcutaneous DF and had serum ferritin below 1mg/l. She was then treated with iv DF 50 mg/kg/24h (via a port-a-cath) but she developed infections and a thrombous and the port-a-cath had to be removed. A heart biopsy at the time of the port-a-cath removal revealed heavy iron siderosis. The chelation treatment was switched to L1 initially at 75 mg/kg/day and then at higher doses to 85 mg/kg/day maximum. During the L1 treatment period serum ferritin levels were reduced within the normal range and the dose was decreased to 50mg/kg/day, but then serum ferritin levels increased again and the dose of L1 increased accordingly and remained at >80 mg/kg/day ever since. In addition to serum ferritin monitoring, which was carried out every 3-5 months, MRI T2 and T2* levels were also monitored approximately every year.
Results and Discussion: With the introduction of L1 the cardiomyopathy was reversed and the patient became asymptomatic with no need for drug therapy for the cardiomyopathy. Serum ferritin levels remained almost within normal range, except in one occasion where an increase was observed when the dose was reduced to 50mg/kg/day. The dose of L1 was then increased and maintained at >80 mg/kg/day, causing serum ferritin levels to be maintained within the normal range. Cardiac and liver MRI T2 and T2* also remained within the normal range in the last 4 years of L1 monotherapy and there have been no toxic side effects.
Conclusion: It would appear that in patients who are not heavily iron loaded, L1 monotherapy at doses >80 mg/kg/day, is sufficiently effective for maintaining normal serum ferritin levels, as well as cardiac and liver iron levels as assessed by MRI T2 and T2*. In this case L1 appears to be superior to DF in clearing excess cardiac and liver iron and to be effective at maintaining normal iron stores.

1] Kontoghiorghes GJ, Kolnagou A. Lancet 2003; 361:184.2] Peng CT et al Eur J Haematol 2003: 70: 392-7. 3] Kolnagou A, et al Brit J Haematol, 2004; 127:360-1. 4] Kolnagou A et al Hemoglobin, 2006;30: 219-27.

NEW APPROACH IN THE DESIGN AND ANTICANCER TARGETING ACTIVITY OF EXPERIMENTAL AND CLINICALLY USED IRON CHELATING DRUGS

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Introduction: Chemicals, biological processes, genetic and other factors are known to cause different forms of cancer. These include environmental, dietary, metabolic, hormonal and immunological factors, viruses, microorganisms and their products and possible combinations of two or more of these.

The role of metal ions: Metal ions play an important role in health and disease, including cancer. All normal and neoplastic cells require essential metal ions such as iron, zinc and copper, metal ion containing proteins, and associated metabolic pathways for growth and proliferation. Under certain conditions some metal ions such as iron and copper can cause DNA damage through the catalytic formation of toxic free radicals and other oxygen activated products. Liver cancer is well documented in iron loaded idiopathic haemochromatosis patients and in copper loaded Wilson's disease patients. Cancer can also be caused by radioactive atoms used in the nuclear industry such as plutonium and uranium, which emit high levels of toxic radiation. Other toxic metals such as cadmium and nickel are also considered to cause cancer through other mechanisms.

The role of chelators: Chelators can be used to modify the chemical, biological and metabolic activity of metal ions and associated proteins or metabolic pathways. Chelating drugs are used for the treatment of more than twenty iron and other metal overloading conditions. Deferoxamine and deferiprone (L1) are used for the removal of iron in transfusional iron loading conditions such as thalassaemia, myelodysplasia and sickle cell anaemia. Penicillamine is used for the treatment of copper overload in Wilson's disease.

In contrast some chelator metal complexes are used for the treatment of cancer eg platinum or gallium complexes, or metal deficiencies eg iron complexes in iron deficiency. Chelator gadolinium complexes are used in magnetic resonance imaging (MRI) and technetium as well as indium in other medical diagnostic techniques.

Many naturally occurring molecules such as ATP, citrate and ascorbic acid have metal binding properties and some proteins such as transferrin and caeruloplasmin have high binding affinity for iron and copper respectively and are used for their transport into cells. Hundreds of proteins participating in the metabolic functions of normal and neoplastic cells bind and incorporate specific metal ions such as iron, copper and zinc, which are essential for their biological activity.

Many common drugs including the anticancer drugs doxorubicin, mitozantrone, bleiomycin and hydroxyurea, have metal chelating properties and their efficacy and toxicity can be modified by the presence of metal ions and chelating molecules.

Conclusion: Within this context, the effect of metal ions and chelators has been investigated in many in vitro, in vivo and clinical experimental models in order to identify molecules and pathways, which can be targeted for the treatment of cancer.

DEFERIPRONE (L1)-25TH YEAR ANNIVERSARY. ITS CLINICAL USE FOR THE COMPLETE TREATMENT OF IRON OVERLOAD AND POSSIBLE USES IN OTHER DISEASES AS MONOTHERAPY OR IN COMBINATION WITH DEFEROXAMINE, DEFERASIROX , DEFERITRIN AND OTHER DRUGS.

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Objective: Effective and affordable chelation therapies are needed for all thalassaemia patients in developed and developing countries. Within this context the cost of the chelation therapy, the efficacy of the established and experimental chelators and the prospect of combination therapies have been examined and compared for the treatment of thalassaemia and other diseases.
Background: Despite many efforts and advances on chelation therapy in the last two decades, there is still a major ethical dilemma with regards to the unavailability of deferoxamine (DF) and deferiprone (L1) to most thalassaemia patients who live in developing countries, because of unaffordable prices. A major initiative is required to change this situation. L1 is inexpensive to synthesise and could become available to all thalassaemia patients worldwide at affordable prices. Several other chelators are being developed but their efficacy, toxicity and availability at low cost is questionable.
Results: There are at least three iron chelators, which are currently being developed for clinical use. One of these is an L1 derivative namely, 1-allyl-2-methyl-3-hydroxypyrid-4-one (L1NAll), which has reached phase I clinical trials. Two other structurally related chelators namely ICL670 (deferasirox) and GT56-252 (deferitrin) have reached phase III and phase II clinical trials respectively. The results so far are promising but do not appear to be significantly better both with respect to efficacy and toxicity by comparison to the established drugs L1 and DF or their combination. Deferasirox has been provisionally approved for clinical use in the US and EU, but although compliance may be high, it is very expensive and toxic in some patients. It has been shown to be effective in increasing faecal but not urinary iron excretion and to reduce liver iron but not to achieve negative iron balance or to reduce cardiac iron levels.
Discussion and Conclusion: As seen with L1 and DF, thalassaemia and other patients are expected to have variable response with respect to the new chelators. Protocols of selecting the most effective and least toxic drug or drug combinations for each patient may increase the therapeutic benefits for patients not responding or experiencing toxicity with a single chelating drug treatment. However, there is no clinical evidence so far to suggest that any of the new experimental chelators will be more effective or less toxic than L1, DF or their combination. Similarly, it is unlikely that any of these will be less expensive than L1. There is an urgent need for the production of inexpensive formulations of L1, which could be used for thalassaemia patients in developing countries, who are not currently receiving any form of chelation treatment.

1] Kontoghiorghes GJ et al Curr Med Chem 2005; 12: 2663-81. 2] Kontoghiorghes GJ. Lancet 2003; 362: 495-6. 3] Kontoghiorghes GJ. Lancet 2005; 366:804.

IRON IN THE SKIN PHYSIOLOGY AND PATHOLOGY

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The peculiarity of iron metabolism, its control, and its role in the physiology of human skin are still poorly understood despite of numerous studies worldwide. It is generally accepted that some skin pathologies such as diabetes- and venous insufficiency-associated ulcers, psoriasis, atopic dermatitis, eszema, infectious skin diseases, acne, etc. demonstrate local as well as generalized imbalance of free radical production/utilization, which is thought to be of relevance for the pathogenesis. The most spectacular examples of free radical-associated skin disorders are psoriasis, atopic dermatitis, and premature UV-induced skin ageing. For the same disorders, significant changes in the levels of iron containing proteins and the expression of transferrin receptors in the skin keratinocytes, fibroblasts, and endothelial cells have been shown. Desferrioxamine and several plant-derived polyphenols with iron chelating properties were found to possess anti-inflammatory and wound healing action. Moreover, keratinocyte proliferation, expression of receptors for inflammatory mediators and their release from the skin cells derived from psoriasis patient were positively affected by selected iron chelators. That allowed to suggesting the feasibility of low molecular iron chelators alone or in combination with plant-derived polyphenols for the topical treatment of psoriasis, atopic dermatitis, acne and the skin hyperpigmentation. Skin equivalent model provides a new investigating system to study the role of iron and free radicals on the structure and functions of keratinocytes, fibroblasts, melanocytes, extracellular matrix and dermal factors such as collagen and basement membrane components, which contribute to cell-cell and cell-matrix interactions. The effects of L1 and/or biotechnologically produced plant-derived phenylpropanoids on the release of pro-inflammatory mediators from keratinocytes and the keratinocyte proliferation (the *in vitro* psoriasis model) were studied. L1 and phenylpropanoids were tested for both the *in vitro* models of melanogenesis and acne. L1 inhibited keratinocyte proliferation and melanogenesis. At the same time, phenylpropanoids were effective as anti-inflammatory agents and inhibitors of tyrosinase and 5-α reductase. We concluded that combinations of L1 and phenylpropanoids with a proper delivery system should be created to achieve high clinical efficacy in the treatment of psoriasis, acne and hyperpigmentation.

DIFFERENTIAL ACTION OF PERMEABLE AND NON-PERMEABLE IRON CHELATORS ON FORMATION OF DINITROSYL IRON COMPLEXES IN VIVO

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Introduction: Formation of dinitrosyl non-heme-iron complexes (DNIC) of the general chemical formula $Fe(RS)_2(NO)_2$ was found in many nitric oxide producing tissues. DNIC signal, characterized by specific EPR spectra, was demonstrated in activated macrophages and their target cells after induction of the high output NO synthase in cancer tissues [2] and in biopsies from acute cardiac allograft rejections. DNIC can also be produced in vitro, if NO, iron and SH-donors are present in the reaction environment. It was suggested that DNIC are an important factor in nitric oxide-dependent cellular processes. Low-mass DNIC might serve a nitric oxide carrier and was associated with NO production in numerous biological systems. DNIC were also demonstrated to act as S-nitrosating intermediates in cells.
Methods: The aim of this study was to investigate the role of cellular labile iron pool in formation of DNIC in erythroid K562 cells. The cells were treated with a nitric oxide donor in the presence of a permeable (SIH) or a lysosomotropic (DFO) iron chelators. DNIC formation was recorded using EPR.
Results: Both chelators inhibited DNIC formation in a dose and time dependent manner. After 6 h of treatment up to 50% inhibition of DNIC formation was observed. To further investigate the role of lysosomal iron in DNIC formation we prevented lysosomal proteolysis by pretreatment of whole cells with NH_4Cl. Pretreatment with NH_4Cl inhibited formation of DNIC in time dependent manner that points to the importance of iron metalloproteins' degradation in DNIC formation in vivo. Fractionation of cell content after treatment with NO donor reveiled that DNIC is formed predominantly in endosomic/lysosomic fraction.
Conclusion: Taken together, these data indicate that lysosomal iron plays a crucial role in DNIC formation in vivo. Degradation of iron containing metalloproteins seems to be important for this process.

LOSS OF MYOFIBERS AND DISRUPTION OF MYOCYTES IS THE POTENTIAL MECHANISM OF IRON OVERLOAD TOXICITY IN CARDIOMYOPATHY IN IRON LOADED THALASSAEMIA PATIENTS. HISTOPATHOLOGICAL AND ELECTRON MICROSCOPY FINDINGS.

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Introduction: Cardiac damage is the most serious toxic side effect of transfusional iron overload, which may lead to cardiac arrhythmias and congestive heart failure. The latter is the most common cause of death in thalassaemia major patients, which occurs mainly after the second decade of life especially in those patients not receiving sufficient or effective chelation therapy. In transfusional iron overload, ferritin and particularly haemosiderin increase substantially in most organs such as the liver, spleen, heart and pancreas resulting in serious damage and abnormal function eg cardiomyopathy.
Patients and Methods: Histopathological studies using differential staining for iron and ultrastructural pathology studies using electron microscopy have been carried out in cardiac biopsies of five thalassaemia patients who suffered congestive heart failure more than seven years ago. All five patients were treated with either sc or iv DF prior to heart failure and four of the patients died. The cardiac biopsy for the electron microscopy study was obtained while removing a thrombus surgically, caused by a port-a-cath for iv DF from a female patient who survived the congestive heart failure,.
Results: Extensive iron deposition and damage in the myocardium has been identified in all the histopathological sections of the cardiac biopsies of the five patients. Excess iron deposition in cardiac cells in the form of ferritin and haemosiderin has also been identified from the ultrastructural pathology studies using electron microscopy. In the cardiac cells the ferritin molecules appeared to form ring structures surrounding the lysosomes, while haemosiderin deposits were shown to accumulate in the cytoplasm. Disruption of lysosomes was evident in some cells. Similarly, there was an apparent loss of myofibers and disruption of myocytes, which seems to be associated with excess iron deposition.
Discussion and Conclusion: These studies suggest that iron toxicity may arise mainly from the incapacity of cells of the heart and other organs to store iron in a safe storage form, resulting in lysosomal rupture and release of proteolytic and other enzymes, which could potentially damage the cells, tissue and the organs involved in the storage of excess iron. Iron toxicity in iron overload may also arise from the presence of labile, toxic iron pools found intracellularly such as a low MWt transit iron pool and low MWt iron released from haemosiderin. Under the same conditions transferrin in the serum is found fully saturated with iron and low MWt non-transferrin bound iron (NTBI) is formed, which can also be a source of toxicity. In all these cases low MWt, labile iron has the potential to catalyse the production of toxic free radicals and the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA etc. Combination of all these factors, including the breakdown of antioxidant and other metabolic controls can also lead to molecular, cellular and tissue damage. As this damage is continuous and not controlled, it may cause a further release of toxic forms of iron from damaged cells and subsequently a vicious circle of increased toxicity and terminal organ damage eg cardiac damage.

GENETIC EPIDEMIOLOGY OF BREAST CANCER; RESULTS OF A POPULATION BASED STUDY IN CYPRUS

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Introduction: In the mid 1990s two BREast CAncer susceptibility genes, namely BRCA1 and BRCA2 were discovered, that predispose to a substantial proportion of familial breast ovarian/cancer patients. Germline mutations in these two highly penetrant genes substantially increase the risk of breast cancer development, characteristically at an early age. Previous studies, characterized a plethora of pathogenic mutations in these two genes, and it is of interest that the type, frequency and penetrance of mutations depend on the population studied. Cyprus has a rather homogeneous population and the aim of this study, was to characterize mutations in these genes and also define the role of the BRCA genes in the breast cancer phenotype in Cypriot families.
Methods: For this study 100 DNAs from patients belonging to 75 Cypriot families with multiple cases of breast/ovarian cancer were selected. Mutation analysis was performed using PCR and sequencing of all exons, as well as intron boundaries of both BRCA genes. Sequencing was carried out using ABI PRISM di-Deoxy Terminator Cycle Sequencing Kit on an ABI 9700 thermal cycler and run on ABI 310 Genetic Analyzer.
Results: Molecular analysis revealed the presence of pathogenic mutations in 14 of the 75 families. These mutations were present in the BRCA1 gene in 4 families and 4 mutations were detected in the BRCA2 gene in 10 families. It is noted that mutation 8984delG in BRCA2 which was present in ten patients from seven different families. Haplotype analysis showed that this was a founder mutation.
Discussion: The crude incidence rate of breast cancer in Cyprus is about 45 cases per 100,000 population, which is similar o other countries in Southern Europe, which makes Cyprus a country with an intermediate breast cancer risk. The present results show that about 15% of familial cases harbour mutations in the BRCA genes. It is of interest that three novel mutations have been identified one in BRCA1 and two in BRCA2. It appears that the BRCA2 gene plays a more substantial role in the breast cancer phenotype of Cypriot families as compared to BRCA1. The identification of a founder mutation in the Cypriot population provides a powerful tool for screening individuals at high risk and also contributes to the early diagnosis of familial cases.

FREE RADICAL CONVERSION OF CATECHOLATES ASSOCIATED WITH CHELATION OF CALCIUM AND OTHER GROUP II METAL CATIONS

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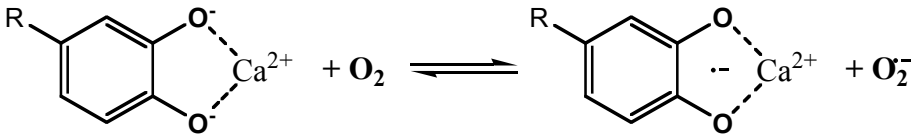
The calcium ion (Ca^{2+}) is a universal intracellular messenger controlling a diverse range of cellular processes such as contraction and secretion, gene transcription and cell growth. The effect of "redox inert" calcium, magnesium, strontium, barium, and zinc on oxidation of pyrocatechol (1,2-benzenediol), dopamine (4-(2-aminoethyl)-4-(2-aminoethyl)pyrocatechol), isoproterenol (4-[1-hydroxy-2-[(1-methylethyl)-amino]ethyl]-1,2-benzenediol), and norepinephrine (4-2-amino-1-hydroxyethyl)-1,2-benzenediol) was studied by measurement of oxygen consumption, EPR-spectroscopy, and by potentiometric titration. Ca^{2+} and Mg^{2+} sharply increased the autooxidation rate at alkaline pH, Sr^{2+} and Ba^{2+} was less active. By contrast, zinc ions promoted deceleration of the catecholate autoxidation. The addition of calcium led to an increase in EPR signal of catecholate semiquinones during autoxidation. Complexing of Zn^{2+} with catecholate ligands resulted in a longer-term spin stabilization. Formation of Ca^{2+}- catecholate complexes led to a decrease in dissociation constants (pK_{ai}) of hydroxyl groups, e.g. pK_{ai} of isoproterenol were 8.8, 10.3, and >12.0 in calcium-free solution and 8.6, 9.9, and 10.7 in the presence of Ca^{2+}. Preferable oxidizability of anion in comparison with neutral form of hydroxyaromatic compounds is well known.



The effect of redox-inert calcium and other alkaline-earth metal ions on the free-radical reactions could be explicated by (i) additional deprotonation of hydroxyl groups of catecholamines with subsequent increase in the rate of electron transfer to O_2, (ii) formation of relatively low stable M^{2+}-*o*-semiquinonate undergoing further oxidation by O_2. Calcium induced oxidoreductive conversion of catecholamines can be a basis for a new concept of the calcium signaling pathways linked with production of reactive oxygen species and semiquinonates.

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IRON CHELATORS AND FREE RADICAL SCAVENGERS AMONG NATURALLY OCCURRING HYDROXYLATED 1,4-NAPHTHOQUINONES

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Multiple 1,4-quinones and 1,4-naphthoquinones possess toxic effect in the cells that is related to cyclic redox reactions and formation of semiquinone as an intermediate. Ortho-hydroxylation sufficiently changes quinoid metabolism, restricts cytotoxicity, and supplies new properties. Echinochrome, 6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoqinone, which diminishes myocardial ischemia/reperfusion injury, and related sea urchin pigments 3-acetyl-2,6,7-trihydroxy-1,4- naphthoqinone (spinochrome C), 2,3,6,7-trihydroxy-1,4-naphthoqinone (spinochrome D), 2,3,6,7- trihydroxy-1,4-naphthoquinone (spinochrome E), and 6-ethyl-2,3,7- trimethoxy-1,4- naphthoqinone (TMEch) scavenge peroxy radicals, trap superoxide radicals, and bind iron ions (see Table).

Table. Antioxidant properties of hydroxylated 1,4-naphthoquinones (HNQ)

HNQ	k_{O2^-*}, $M^{-1}\cdot s^{-1}$	C_{inhFe}, μM	k_{ROO*}, $M^{-1}\cdot sec^{-1}$	pK_i	
echinochrome	$6.4\cdot10^5$	6	$4.0\cdot10^4$	5.30	7.64
spinochrome D	$3.4\cdot10^5$	30	$1.4\cdot10^5$	6.07	8.45
spinochrome C	$1.7\cdot10^5$	5	$1.7\cdot10^5$	6.52	7.57
spinochrome E	-	5	$2.5\cdot10^5$	5.84	7.30
TMEch	$2.6\cdot10^4$	1000	not active	8.17	8.85

Dissociation constants (pK_i) were determined by methods of potentiometric titration in 40% EtOH. The rate constant (k_{ROO*}) of HNQ – ROO* reaction was determined in a model of free radical (AIBN)-initiated oxidation of cumene. The rate constant (k_{O2^-*}) of HNQ - O_2^-* reaction was determined in xanthine/xanthine oxidase system by a competitive method using nitro blue tetrazolium. The HNQ at [C_{inhFe}] caused a 10-fold decrease in the ferrous/ascorbate initiated liposomal lipid peroxidation.

All tested HNQs but not TMEch were more effective in inhibition of the Fe^{2+}/ascorbate initiated liposome peroxidation than that initiated by heme iron (hemin). UV/VIS spectra directly evidenced formation of HNQ/Fe^{2+} (HNQ/Fe^{3+}) complexes. In isolated sarcoplasmic reticulum, echinochrome protected the ATP-dependent Ca^{2+}-pump system from being damaged by Fe^{2+}/ascorbate, i.e., it prevents the oxidation caused by Fenton/Haber-Weiss chemistry in biomembranes. We conclude that the HNQ containing hydroxyl groups in the 2nd and 3rd positions may act as powerful radical scavengers and iron chelators. The mechanisms underlying the antioxidant and iron chelating activity of echinochrome and spinochromes are similar to that of known *o*-hydroxylated aromatic compound, including large numbers of plant flavonols.

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CARDIAC PROTECTION OF NON-MITOGENIC HUMAN ACIDIC FGF FROM OXIDATIVE DAMAGE *IN VITRO* AND *IN VIVO*: POTENTIAL APPLICATION FOR THE PREVENTION OF DIABETIC CARDIOMYOPATHY

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To study the cardiac protection of non-mitogenic human acidic fibroblast growth factor (nm-haFGF) against oxidative injury in vitro and in vivo, neonatal ventricular cardiomyocytes were exposed to hydrogen peroxide at different concentrations with and without the presence of nm-haFGF. Exposure of cardiomyocytes to hydrogen peroxide caused a significant cytotoxic effect and nm-haFGF processes a significant protection from hydrogen peroxide. Cardiomyocytes that were genetically overexpressed nm-haFGF were significantly resistant to hydrogen peroxide-induced cytotoxicity, as measured by cell viability. Hearts from rats pre-treated with saline, haFGF, or nm-haFGF for 24 h were subjected to ischemia/reperfusion (I/R) in Langendorff system. I/R induced a cardiac dysfunction in the saline group, but not in haFGF- or nm-haFGF-pre-treated group. I/R also caused a release of cardiac enzyme LDH into and an increase in lipid peroxide contents in the effluxes of the hearts from saline-treated rats, but not from haFGF- or nm-haFGF-pre-treated rats. There was no difference for the cardio-protective effects between haFGF and nm-haFGF. These results suggest that nm-haFGF, like the native haFGF, provides a significantly cardiac protection from oxidative damage *in vitro* and *in vivo*. Since the major mechanisms for diabetic cardiomyopathy is oxidative stress, and diabetes also decrease various growth factors in the heart, the potential application of nm-haFGF to diabetic patients for preventing the cardiomyopathy will be discussed.

SKELETAL FREE RADICALS MEDIATED BY IRON: A NEW THERAPEUTIC TARGET OF POSTMENOPAUSAL OSTEOPOROSIS USING ORAL CHELATION

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Introduction: It is believed that an increase in iron content is associated with an increased likelihood of oxidative damage at the point of iron accumulation. However, to our knowledge, no direct evidence has shown that the iron accumulated in skeletal tissue causes free radical oxidative damage, and consequent bone loss. An *in vivo* study on iron depletion from skeletal tissues of chelator (1-N-Docosyltriethylenetetraaminepentaacetic acid)-treated ovariectomized (OVX) rats was conducted to examine the relationship between iron and the oxidative damage, and the linkage of the damage with bone loss in the rats.

Methods: OVX rats (six months of age) were treated with the chelator three times a week for 9 weeks through oral gavage (100 μmole/kg). Then, the iron and LLOFR (long-lived organic free radical) contents of the bone were assayed with EPR ((Electron Paramagnetic Resonance) spectroscopy. Also, bone Mineral Density (BMD) and bone micro-structural indices were quantitatively analyzed by μ-CT (Micro-Computered Tomography).

Results: Iron and LLOFR levels increase significantly in the bone of OVX rats compared with Sham controls. However, chelator-treated OVX rats have significantly lower iron and LLOFR contents than that of OVX controls as well as Sham controls. The LLOFR content is positively proportional to iron levels. Importantly, the bones of chelator-treated OVX rats have higher BMD compared with OVX controls. Also, bone micro-structural indices have indicated that the chelator-treated OVX rats have better bone quality than that of OVX controls.

Discussion and conclusion: The results reveal that chelation treatment can slow or perhaps even reverse the iron accumulation and as a result, prevent oxidative damage in the bone in the OVX rats. Our studies not only show that increased iron in the bone is associated with the increase of LLOFR, but also demonstrate that iron is a key factor in LLOFR formation and that it can be inhibited by iron depletion using chelation. Significantly, osteopenic development in OVX rats is mitigated as iron accumulation and iron associated oxidative damage are prevented. Thus, our findings may lead to new therapeutic approaches to address a number of aging diseases that are or may be associated with iron accumulation and iron associated oxidative damage.

LIGHTS AND SHADOWS IN THE CHELATION TREATMENTS

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New insights on chelation treatment have been published, recently. However, many unanswered questions remain. For instance, in the paper by Borgna-Pignatti et al. besides the different length in the time treatment between Deferiprone (DFP) vs Deferoxamine (DFO)-group, other concerns include 1) the report by Hoffbrand et al. of 4/51 deaths (three thalassemia major patients and one sideroblastic congenital anemia patient) from congestive heart failure after a mean of 18.7 months (range, 4 – 35 months) of DFP treatment was not addressed 2) attributing cardiac events in six patients (one after 20 months and five after three years of discontinuing DFP) to DFO, despite the enhanced survival following treatment with this chelator previously reported by these authors. Additionally, the definition of "cardiac events" should refer only to major events such as death or hospitalization due to cardiovascular causes. Finally, the unconfirmed suggestion of DFP liver toxicity raises the possibility that the participating centers selected more severe cases for the DFO group. This was the case for 71 DFP-treated patients in our multicenter randomized clinical trial. The centers, some of which also participated in cardiac morbidity and mortality survival study, switched all patients to DFO after a year of DFP treatment.

In the Pennell et al. paper, the DFP dose was higher (92 mg/Kg/d) than recommended by the manufacturer (75 mg/Kg/d).This raises questions about the applicability of the result. Also, the improvement of 3.1% in the Left Ventricular Ejection Fraction (LVEF) at 12 months (DFP: 69.7-72.8%, P=<.001, DFO: 68.4- 68.7%, P=.66) is within the normal range, and is not clinically significant. It is known that, thalassemia patients have higher ejection fraction values than nonanemic subjects. The increment in LVEF was most likely due to hemodynamic variables as a consequence of high cardiacoutput due to more severe anemia, rather than an improvement in cardiac function due to removal of cardiac iron. Lower Hb levels in the DFO-treated group (P=0.023) and the significant reduction in end-systolic but not end-diastolic volume support this argument. Also the clinical significance, in terms of heart iron removal, of a 1.5 ms increase in the myocardial T2* signal is doubtful as it is suggested by the cardiac iron concentration exponential curve derived from a gerbil model of iron cardiomyopathy. Finally, the correlation between the myocardial T2* signal and cardiac function for a wider T2* signal range is controversial.

Cappellini et al. suggested that despite the ICL670 safety profile, the primary end-point of the trial, maintenance or reduction of Liver Iron Concentration (LIC), was obtained only in the group of patients with LIC >14 mg Fe/gr/dw that received 30 mg/kg of ICL670. They declare that in the other group of patients with medium and low LIC the primary end-point was not obtained because of lower ICL670 dosage used in comparison with DFO. These findings suggest that, so far, it is not known if higher doses of ICL670 can control iron overloading in patients with low or medium LIC without compromising safety. On the contrary, as it was shown previously in an independent, multicenter randomized clinical trial, DFP was safe and able to control iron overloading in thalassemia major patients with a low or medium iron body burden after one year of treatment.

In conclusion, additional clinical research aimed at clarifying these points is necessary.

A RANDOMIZED CONTROLLED TRIAL COMPARING THE COMBINATION THERAPY OF DEFERIPRONE (L1) AND DESFERRIOXAMINE (DFO) VERSUS L1 OR DFO MONOTHERAPY IN PATIENTS WITH THALASSEMIA MAJOR

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Several clinical studies have shown that the combination of deferiprone (L1) with desferrioxamine (DFO) is efficacious with regard to decrease in serum ferritin (SF), reduction of liver iron concentration (LIC) and increase in urinary iron excretion (UIE) in patients suffering from thalassemia major. However, there is a lack of randomized controlled trials comparing changes of LIC in patients treated with the combination therapy versus patients treated with L1 or DFO monotherapy. This randomized controlled trial aimed at analyzing and comparing the efficacy and safety of the combination therapy of L1 and DFO versus monotherapy with either L1 or DFO in patients with thalassemia major during a study period of one year.

A total of 95 patients with thalassemia major were randomized into one of the following 3 treatment arms: L1 was given orally at a daily dose of 75 mg/kg either alone, in combination with s.c. DFO (40-50 mg/kg twice weekly) or DFO was given alone at a dose of 40-50 mg/kg 5 days a week (control arm). All patients had been treated with DFO prior to the study.

Liver iron concentration (LIC) was measured in liver biopsies taken at baseline and after one year. Biochemistry measurements including serum ferritin (SF) and liver enzymes were performed at 3-monthly intervals. Blood counts were analyzed weekly for 8 weeks and thereafter bi-weekly. Cardiac function (ECHO) was assessed 6-monthly. The average urinary iron excretion (UIE) of weeks 1, 12, 26, 38 and 54 was calculated. In patients receiving combination therapy, UIE was measured on two different days, i.e. during L1 monotherapy and combination therapy.

Compliance was excellent in all three treatment arms. Only 14% of patients assigned to the DFO control arm and 11% of patients randomized to the combination treatment arm were non-compliant. In total, fourteen patients (15%) dropped out from the study: one due to biopsy failure at baseline, five withdrawals from informed consent, four non-compliance to treatment, one due to jaundice, one died from arrhythmia induced heart failure at study start, and two developed agranulocytosis. The most common adverse events were transient increase in liver enzymes, nausea and arthralgia. The most serious adverse event associated to L1 therapy was agranulocytosis observed in 2 patients (2.1%).
The average change in LIC after one year was most pronounced in the combination arm (-47%; p=0.001), but LIC was also significantly lowered after one year monotherapy with L1 (-23%; p=0.13) or DFO (-45%; p=0.003). The majority of patients in all treatment arms showed a clear decrease in SF and the mean SF was significantly reduced in the combination arm after one year (-2'120µg/L; p=0.0003). The left ventricular ejection fraction increased during combination therapy (+3.4% absolute units; p=0.19), whereas it slightly decreased (-2.1% absolute units; p=0.41 and p=0.55, respectively) after either L1 or DFO treatment. The mean daily UIE was higher in L1-containing regimens than with DFO single agent therapy. UIE on days of combination (0.90±0.33 mg/kg/24h) was significantly higher than on days of L1 monotherapy (0.53±0.26 mg/kg/24h) (p=0.0003).

This study indicates that the combination of daily L1 and twice weekly DFO at standard doses is a highly efficacious and safe chelation therapy for patients with thalassemia major.

DO IRON CHELATORS INFLUENCE PROGRESSION OF ATHEROSCLEROSIS?

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Epidemiological studies and experimental data suggest iron involvement in atherosclerosis. Meanwhile, after numerous epidemiological studies (many of them performed in Utrecht) the relation between iron and atherosclerosis remains contradictory, due to the complexity of this relation and problems and flaws in methodology. Some results of recent epidemiological investigations will be presented.
We investigated the mechanism by which iron may stimulate atherogenesis. Endothelial activation induces expression of vascular adhesion molecules and of endothelial inflammatory chemokines. Initial adhesion by selectins mediates a rolling interaction, followed by a firm attachment by means of integrins. Adherent monocytes migrate into the subendothelial space under the influence of chemoattractant molecules, and differentiate into foam cells (oxLDL loaded macrophages). We have shown that iron can induce early functional and structural vascular abnormalities due to endothelial dysfunction, which is associated with the subsequent induction of oxidative stress. Iron *in vitro* upregulated IL-6 production by endothelial cells, while iron chelators inhibited upregulation of endothelial adhesion molecule expression. The most important molecular species of iron in plasma that is responsible for increasing interaction of monocytes and endothelial cells is non-transferrin bound iron (NTBI). We have demonstrated that NTBI is not only present in severe iron overload but also in HFE heterozygotes. The effects of NTBI are intracellular; involve production of intracellular oxygen radicals and subsequent increased expression of adhesion proteins on endothelial cells and monocytes. The radicals may be involved in the regulation of transcription factors, such as NF-κB, regulating expression of adhesion molecules and inflammatory cytokines. All biological effects can be abolished by addition of iron chelators. The observed effects could, at the long term, promote formation of atherosclerotic lesions. They might, however, also promote movement of monocytes (as well as of PMN) to infectious lesions, and therefore improve innate immunity. Iron chelators will interfere with such processes.

IRON REGULATORY PROTEINS: ROLE IN HEALTH AND DISEASE

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Cellular iron homeostasis is achieved by the coordinate and reciprocal post-transcriptional regulation of transferrin receptor 1 and ferritin expression, which are key molecules for iron acquisition and storage, respectively. The mechanism involves interactions between cytoplasmic iron regulatory proteins (IRP1 and IRP2) and iron responsive elements (IREs) located within non-coding sequences of TfR1 and ferritin mRNAs. In iron-deficient cells, IRE/IRP interactions stabilize TfR1 mRNA and inhibit the translation of the mRNAs encoding H- and L-ferritin. The RNA-binding activities of IRP1 and IRP2 are induced in iron deficiency. In iron-replete cells, IRP1 assembles a cubane [4Fe-4S] iron sulfur cluster (ISC), which prevents the binding to IREs and converts the protein to a (cytosolic) c-aconitase. By contrast, IRP2 undergoes ubiquitination and degradation by the proteasome. IRPs respond not only to cellular iron levels but also to additional stimuli, such as oxygen levels, oxidative stress and nitric oxide. The targeted disruption of both IRP1 and IRP2 is associated with early embryonic lethality, underlying the physiological significance of the IRE/IRP regulatory system. IRP1$^{-/-}$ mice express increased ferritin levels in the kidney and brown fat without showing any pathological abnormalities. On the other hand, IRP2$^{-/-}$ mice misregulate iron metabolism in the duodenum, the CNS and the erythron and exhibit microcytic anemia. A gene targeting approach has also yielded IRP2$^{-/-}$ mice that progressively accumulate iron in the CNS and develop a severe neurodegenerative disease. Further development and characterization of animal models is expected to shed more light on the specific functions of each IRP *in vivo*.

IRON-DEPENDENT DEGRADATION OF APO-IRP1 BY THE UBIQUITIN-PROTEASOME PATHWAY

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Introduction: Iron regulatory protein 1 (IRP1) controls the translation or stability of several mRNAs by binding to "iron responsive elements" (IREs) within their untranslated regions. Its activity is regulated by an unusual iron-sulfur cluster (ISC) switch. Thus, in iron-replete cells, IRP1 assembles a cubane ISC that inhibits RNA-binding activity and converts the protein to cytosolic aconitase. The purpose of the study is to identify and characterize new modes for IRP1 regulation.
Results: We show that the constitutive $IRP1_{C437S}$ mutant, which fails to form an ISC, is destabilized by iron. Thus, exposure of H1299 cells to ferric ammonium citrate reduced the half-life of transfected $IRP1_{C437S}$ from ~24 h to ~10 h. The iron-dependent degradation of $IRP1_{C437S}$ involved ubiquitination, required ongoing transcription and translation and could be efficiently blocked by the proteasomal inhibitors MG132 and lactacystin. Similar results were obtained with overexpressed wild type IRP1, which predominated in the apo-form even in iron-loaded H1299 cells, possibly due to saturation of the ISC assembly machinery. Importantly, inhibition of ISC biogenesis in HeLa cells by siRNA knock-down of the cysteine desulfurase Nfs1, sensitized endogenous IRP1 for iron-dependent degradation.
Conclusion: These data uncover an alternative mechanism for the regulation of IRP1 abundance as a means to control its RNA-binding activity, when the ISC assembly pathway is impaired.

THE CARDIAC FUNCTION IMPROVEMENT OF THALASSEMIA PATIENTS IN USING DEFERIPRONE (L1)

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Deferoxamine (DFO) therapy has been associated with improved survival of thalassemia patients. However, cardiac disease remains the main cause of death in those patients. Deferiprone (L1) is currently one of the oral chelation agents used as an alternative to DFO. Both DFO and L1 have demonstrated their ability to normalize cardiac function in patients with iron-induced cardiac disease and evidence shows that L1 is more effective in cardiac iron removal. The removal of excess myocardial iron by continuous i.v. DFO infusions in patients with iron-induced cardiac failure is limited by its molecular size (lesser cellular penetration). A possible explanation for the beneficial effect of DFO is that it may be related to extracellular events such as the decline in the plasma concentration of non-transferrin-bound iron (NTBI). The improvement in cardiac function that can be observed during L1 therapy may have several mechanisms. Because of its tiny size and physio-chemical characteristics, it can readily penetrate iron-loaded myocytes where it may exert anti-oxidant activity, or bind the excess iron and carry it out of the cell into the circulation where it is excreted mainly in the urine. In addition, L1 cardioprotective effects may be related to its ability to mobilize citrate-bound iron or other forms of NTBI. The need to maintain continuous levels of chelators in the plasma to decrease the cardiac injury by toxic species of iron may explain, in part, the different cardiac protective effects observed between continuous 24-hour infusion and the standard regimen of DFO. This need could also explain the difference in cardio-protection observed between standard s.c. DFO administration and uninterrupted L1 therapy. Non-invasive techniques of quantification of the iron burden using MRI have been validated. We have continued to explore the use of readily available bed-side tools, using echocardiograms and biochemical markers of cardiac dysfunction to monitor thalassemia patients with cardiac complications. We conducted a clinical study comparing L1 with DFO. L1 chelation had marginal benefits in increasing cardiac function and reducing cardiac iron accumulation. Using SI of MRI and LVEF to monitor cardiac systems, in particular the cardiac functions that are closely associated with iron overload-related complications and mortality, proved to be practical. The relevance of standard cardiovascular medications to this population group has yet to be established with clarity; nevertheless our increased understanding of the cardiac paraphysiology and improved ability to detect at risk populations is yielding improved outcomes and reduced morbidity in our patients.

RECENT ADVANCES IN PHYSIOLOGY AND PATHOPHYSIOLOGY OF IRON METABOLISM

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Iron (Fe) is a vitally important element metabolism because of its unsurpassed versatility as a biologic catalyst. However, when not appropriately shielded or when present in excess, Fe plays a key role in the formation of extremely toxic oxygen radicals, which ultimately cause peroxidative damage to vital cell structures. Because of the inherent danger of Fe, specialized molecules for the acquisition, transport (transferrin [Tf]), and storage (ferritin) of Fe in a soluble non-toxic form have evolved. Delivery of Fe to most cells occurs following the binding of $Fe^{3+}{}_2$-Tf to Tf receptors on the cell membrane. The Tf-receptor complexes are then internalized by endocytosis, and Fe is released from Tf by a process involving endosomal acidification. Iron is then transported through the endosomal membrane by the Fe^{2+} transporter DMT1. Because the substrate for DMT1 is Fe^{2+}, reduction of Fe^{3+} must occur in endosomes; a plausible candidate for endosomal ferrireductase is Steap3, whose defect is responsible for impaired Fe uptake by erythroid cells from *nml054* mouse mutants. Following its escape from endosomes, iron is transported to intracellular sites of use and/or storage in ferritin, but this aspect of Fe metabolism, including the nature of the elusive intermediary pool of Fe and its cellular trafficking, remains enigmatic. Importantly, DMT1 is also involved in the absorption of inorganic Fe in the duodenum. Cells are also equipped with a regulatory system that controls Fe levels in the labile pool. Levels of iron modulate the capacity of iron regulatory proteins (IRPs) to bind to the iron responsive elements (IREs) present in the untranslated regions of mRNAs for several proteins involved in Fe metabolism (e.g., ferritin, transferrin receptor, DMT1); these associations, or lack of them, in turn control the expression of these proteins. Organisms and cells have limited ability to excrete excess Fe and only some specialized cells have active mechanisms to export Fe. Iron release from these "donor cells" (enterocytes and macrophages that recycle hemoglobin Fe) is mediated by ferroportin. Ferroportin is the receptor for hepcidin, a polypeptide hormone made by the liver in response to Fe stores and inflammation. Binding of hepcidin, the principal iron regulatory hormone, to ferroportin leads to the internalization and degradation of ferroportin.

'CAGED-IRON CHELATRS' AS POWERFUL PRODRUGS TO PROTECT THE SKIN CELLS AGAINST IRON-MEDIATED LYSOSOMAL DAMAGE AND NECROTIC CELL DEATH

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Introduction: The most important cellular pool of redox-active labile iron (LIP) exists within lysosomes, making these organelles vulnerable to oxidative stress. Our previous studies with skin cells have demonstrated that Ultraviolet A (UVA, 320-400 nm), the oxidizing component of sunlight, triggers damage to lysosomal membranes leading to leakage of potentially harmful proteases and labile iron into the cytosol, which in turn exacerbates the overall damaging process in the cells and ultimately causes necrotic cell death [1-3]. The chelation of intra-lysosomal LIP by strong iron chelators efficiently prevents lysosomal rupture and the ensuing cell death induced by UVA [1-2] but chronic use would cause severe side effects due to systemic iron depletion. Prodrugs that become activated in skin cells at physiologically relevant doses of UVA, such as 'caged-iron chelators', may provide dose and context dependent release. We have recently synthesized prototypical iron chelator compounds derived from salicylaldehyde isonicotinoyl hydrazone (SIH) and pyridoxal isonicotinoyl hydrazone (PIH) and demonstrated that the intracellular LIP and subsequent necrotic cell death of human skin fibroblasts is significantly decreased upon exposure to a combination of the prototypical compounds and physiologically relevant UVA doses [4]. In the present study we investigated further the mechanism of action of the caged-iron chelators by monitoring the stability of lysosomal membrane upon UVA irradiation following treatment of skin fibroblasts with these compounds.
Methods: The human primary skin fibroblast cells FEK4 cells were first irradiated with a range of doses of UVA at natural exposure levels in presence or absence of caged-compounds. Three independent assays were then used to monitor the integrity of lysosomal membranes following UVA irradiation of FEK4 cells: (1) Fluorescence microscopy using an acidotropic fluorescent probe, Lysosensor green DND-189. (2) Immunocytochemistry to check the extent of delocalisation of the lysosomal Cathepsin B protease to the cytosol as a result of radiation-induced damage to these organelles. (3) Spectrophometric Neutral Red uptake assay to quantify the extent of lysosomal damage and protection provided by the compounds.
Results: Our results demonstrate that unlike the parent compounds, the caged iron chelators do not affect the intra-lysosomal LIP, until exposure to a physiologically relevant UVA dose when they subsequently provide promising levels of protection for skin fibroblast cells against UVA-mediated lysosomal damage and necrotic cell death.
Conclusion: This novel light-activated prodrug strategy may therefore be used to protect skin cells against the deleterious effects of sunlight.
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ECG CHANGES IN THALASSEMIA PATIENTS: CORRELATIONS WITH MYOCARDIAL IRON OVERLOAD AND MYOCARDIAL FIBROSIS

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Background: Non-specific electrocardiogram (ECG) changes are associated to thalassemia major (TM) but relationships between ECG changes and pathologic cardiac substrates were not investigated. Cardiovascular Magnetic Resonance (CMR) allows to evaluate myocardial iron overload (MIO) and myocardial fibrosis.
Objective: To assess whether ECG might pre-screen patients before CMR we correlated ECG changes with MIO and myocardial fibrosis evaluated by CMR in TM patients.
Materials and Methods:. MIO of 72 females and 32 males with TM (27 yy ± 8) was evaluated by multislice multiecho CMR T2*: global T2* value of the myocardium and T2* values for all the standardized 16-segments of the heart model were calculated. Myocardial fibrosis was evaluated by late gadolinium-enhanced acquisitions. ECG was performed within one month from CMR and read blindly by 2 cardiologists.
Results: The inter-rate (K) agreement between the 2 cardiologists was 0.8. ECG changes were read in 45% of cases. T2* values were normal (≥ 20 ms) for all 16 segments in 21% TM patients; out of the patients with abnormal segmental T2* values (< 20 ms), 29 % had a homogeneous MIO; 21% of TM patients showed myocardial fibrosis. A significant correlation was found between abnormal T2* global values (< 20 ms) and changes in ECG (chi-square 3.9; P = 0.04). Patients with abnormal ECG had lower T2* global values and less segments with normal T2* values compared to patients with normal ECG (19 ± 11 ms vs 27 ± 13 ms, P = 0.001; 6 ± 6 segments vs 10 ± 6 segments, P = 0.003). A significant correlation was found between the presence of myocardial fibrosis and changes in ECG (chi-square 3.6; P = 0.05). Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of ECG in detecting abnormal T2* global values were 54%, 67%, 51% and 70%, respectively; in detecting myocardial fibrosis were 66%, 60%, 31% and 87%, respectively; in detecting MIO and/or myocardial fibrosis were 61%, 72%, 42% and 81%, respectively.
Conclusions: ECG changes in TM patients correlated significantly with MIO, myocardial fibrosis and MRI pathological findings (MIO and/or myocardial fibrosis). Due to its good PPV and low cost, ECG could be a suitable guide to orient to CMR exam in TM.

MYOCARDIAL IRON OVERLOAD AND MYOCARDIAL FIBROSIS IN THALASSEMIA INTERMEDIA VERSUS THALASSEMIA MAJOR PATIENTS: A COMPARATIVE MULTICENTER STUDY

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Background: Due to chronic anemia, resulting in high cardiac output state, and iron overload, mainly related to increased gastro-intestinal iron absorption, cardiac involvement is expected in thalassemia intermedia (TI) patients.
Objectives: To assess whether Cardiac magnetic resonance (CMR) might be as good in TI as in TM patients, as a tool for monitoring and management, we correlated CMR data on myocardial iron overload and fibrosis, with CMR morphological and functional cardiac parameters in a TI and a TM patients pools.
Methods: In 60 TI and 60 TM patients myocardial and liver iron overload (MIO) were assessed by T2* sequences; atrial areas and biventricular function parameters were obtained from Cine sequences; myocardial fibrosis was evaluated by late gadolinium-enhanced acquisitions.
Results: In TI patients MIO was present in 23%; of them 92% had a heterogeneous distribution. Abnormal liver T2* values were detected in 72% of cases; patients with myocardial fibrosis (32%) showed significantly reduced or borderline left ventricular ejection fraction (LVEF) (P=0.01); there was no correlation between global heart T2* values and biventricular EF, myocardial fibrosis or liver T2*. LV and right ventricular (RV) dilatation with increased stroke volume was present in 32% and in 13% of TI patients, respectively. All showed normal or increased LVEF and RVEF in 71% and in 98% cases, respectively, while 60% showed bi-atrial enlargement. Mean Hb-levels and MIO were significantly lower than in TM patients ($P < 0.0001$ and $P < 0.0001$); TI patients with homogeneous MIO was 9% while TM patients were 40% ($P = 0.11$). TI patients had heterogeneous MIO with normal T2* (≥ 20 ms) global value more frequently than TM patients ($P = 0.01$). Liver T2* values were similar in the 2 groups ($P = 0.17$). End-diastolic volume indexes, stroke volumes, EF and bi-atrial areas were significantly higher in TI patients than in TM; presence of myocardial fibrosis was not significantly different ($P = 0.9$).
Conclusions: CMR confirms its role as suitable guide to cardiac managements in TI, as well as in TM. TI patients showed lower myocardial iron burden and more pronounced high cardiac output findings. The high cardiac output state seems to increase the relative risk of further dilatation, myocardial fibrosis, and decreased systolic function. Thus, protocols for blood transfusions and chelation therapy in this disease might be revised.

RECENT ADVANCES IN THE DEVELOPMENT OF CANCER THERAPEUTICS IN RELATION TO IRON METABOLISM

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Introduction: Novel chemotherapeutics with marked and selective anti-tumor activity are essential to develop, particularly those that can overcome resistance to established therapies. Iron (Fe) is critical for cell cycle progression and DNA synthesis and potentially represents a novel molecular target for the design of new anti-cancer agents. The aim of this current study was to evaluate the anti-tumor activity and Fe chelation efficacy of a new class of Fe chelators using human tumors, namely the d-2-pyridylketone thiosemicarbazone (DpT) series of ligands.
Materials and Methods: Human tumor xenografts in nude mice. RT-PCR and Western analysis.
Results and Discussion: In this investigation [1], the ligands showed broad anti-tumor activity in 28 different tumor cell lines and could overcome resistance to established anti-tumor agents. The *in-vivo* efficacy of the most effective chelator identified, Dp44mT, was assessed using a panel of human xenografts in nude mice. These tumors include melanoma, lung carcinoma, ovarian carcinoma and neuroepithelioma. After 7 weeks, net growth of a melanoma xenograft in Dp44mT-treated mice was only 8% of that in mice treated with vehicle. In addition, no differences in these latter animals were found in hematological indices between Dp44mT-treated mice and controls. No marked systemic Fe-depletion was observed comparing Dp44mT- and vehicle-treated mice, probably due to the very low doses (0.4-0.75 mg/kg/day) required to induce anti-cancer activity. Dp44mT caused up-regulation of the Fe-responsive tumor growth and metastasis suppressor, *N-myc downstream regulated gene-1*, *Ndrg1*, in the tumor but not the liver, indicating a potential mechanism of selective anti-cancer activity.
Conclusion: These results indicate that the novel Fe chelators have potent and broad anti-tumor activity and can overcome resistance to established chemotherapeutics due to their unique mechanism of action.

1] Whitnall, M., Howard, J. Ponka, P. and Richardson, D.R. (2006) A novel class of iron chelators with a wide spectrum of potent anti-tumor activity that overcome resistance to chemotherapeutics. *Proc. Natl. Acad. Sci. USA* (In Press, Accepted Aug 3).

NITROGEN MONOXIDE (NO)-MEDIATED IRON MOBILIZATION FROM CELLS IS LINKED TO NO-INDUCED GLUTATHIONE EFFLUX VIA MRP1

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Introduction: Nitrogen monoxide (NO) plays a role in the cytotoxic mechanisms of activated macrophages against tumor cells by inducing the release of the essential metal, iron (Fe). We showed that NO-mediated Fe efflux from cells required glutathione (GSH) (Watts, R.N. and Richardson, D.R. (2001) *J. Biol. Chem*. 276:4724) and have hypothesized that a GS-Fe-NO complex is released. Hence, we studied the role of the GSH-conjugate transporter, multi-drug resistance-associated protein 1 (MRP1), in NO-mediated Fe efflux.

Materials and Methods: MCF7 wild-type cells and MCF-7VP cells hyper-expressing MRP1. Cells were labeled with ^{59}Fe-transferrin and then reincubated with NO donors.

Results and Discussion: In this study [1], we showed the MCF7-VP cell line over-expressing MRP1, exhibited a 3-4-fold increase in NO-mediated ^{59}Fe and GSH efflux compared to wild-type cells (MCF7-WT) over 4 h. The NO-mediated ^{59}Fe and GSH efflux was significantly decreased by the GSH-depleting agent and MRP1 transport inhibitor, L-buthionine-[*S,R*]-sulfoximine (BSO). Furthermore, other MRP1 inhibitors, MK571, probenecid and difloxacin, significantly inhibited NO-mediated ^{59}Fe release. Comparable results were observed for the MRP1 over-expressing cell line, but not for those expressing another drug efflux pump, P-glycoprotein. EPR spectroscopy demonstrated that the characteristic dinitrosyl-dithiol-Fe complex (DNIC) peak in NO-treated cells was increased by the MRP1 inhibitors, probenecid and MK571. This observation indicated inhibited DNIC transport from cells. Further, MCF7-VP cells were more sensitive than MCF7-WT to the growth inhibitory effects of NO, and this was potentiated by GSH-depletion using BSO. These data indicate the importance of GSH in NO-mediated inhibition of proliferation.

Conclusions: Collectively, NO stimulates Fe and GSH efflux from cells via MRP1. Active transport of NO by MRP1 overcomes diffusion that is inefficient and non-targeted and this has broad ramifications for understanding NO biology.

[1] Watts, R.N., Hawkins, C., Ponka, P. and Richardson, D.R. (2006) Nitrogen monoxide (NO)-mediated iron mobilization from cells is linked to NO-induced glutathione efflux via MRP1. Proc. Natl. Acad. Sci. USA 103(20):7670-7675.

MITOCHONDRIAL-PERMEABLE IRON CHELATORS PREVENT CARDIAC HYPERTROPHY IN THE MOUSE MODEL OF FRIEDREICH'S ATAXIA

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Introduction: There is no effective treatment for the severe cardiomyopathy and neurological deficits that occur in the most common autosomal recessive disease, Friedreich's ataxia (FA). The identification of potentially toxic iron (Fe) deposits in the mitochondria of patients with FA suggests that Fe may play a role in its pathogenesis due to cytotoxic radical generation. We examined if an Fe chelator that permeates the mitochondrion can prevent the pathology observed in a mouse model of FA, namely the Frda/MCK conditional frataxin knockout mouse (Puccio, H et al. (2001) *Nature Genetics* 27:181-6). This model reproduces the cardiac pathology observed in FA, including myocardial hypertrophy and mitochondrial Fe-loading.

Materials and Methods: Frda/MCK conditional frataxin knockout mouse, histological analysis, inductively coupled plasma mass spectrometry.
Results and Discussion: In these studies, the lipophilic and mitochondrion- permeable ligand, pyridoxal isonicotinoyl hydrazone (PIH), was used in combination with the hydrophilic chelator, desferrioxamine (DFO). Iron chelation slowed total body weight loss in Frda/MCK frataxin knockout mice. Significantly, treatment with the chelators markedly inhibited the development of myocardial hypertrophy and deposition of myocardial Fe deposits. While chelation removed excess Fe from cardiac tissue, it did not negatively impact on hematological indices. Our study shows that Fe chelation therapy prevented the cardiac hypertrophy observed in Frda/MCK mice. These results indicate that mitochondrial Fe deposition is important in the pathogenesis of FA.

Conclusions: Collectively, the results suggest that alterations in myocardial Fe metabolism play a role in the pathogenesis of FA and that mitochondrial permeable Fe chelators may be a useful therapeutic strategy.

NEW DEVELOPMENTS IN TRIVALENT CATION CHELATORS: EXTRAFUNCTIONALIZATION AND LIGAND-COMBINED STRATEGIES

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The development of new chelating agents for "hard" metal ions, in particular, for iron(III) and the group 13 of metal ions has been an important goal in medicinal chemistry due to their potential application, either for controlling situations of metal toxicity in the body (e.g. Fe and Al) or for clinical radiodiagnostic (e.g. $^{67/68}$Ga, ^{111}In). However, for the design of chelating drugs for clinical application it is paramount to consider the properties governing not only the metal selectivity but also those related with the *in vivo* transport and molecular recognition by the target disease sites. Among the *O*,*O*-donor ligands, the 3-hydroxy-4-pyridinones (3,4-HP) have attracted considerable attention due to their high affinity for hard metal ions, *in vivo* stability as well as facile modifiability to tune the major parameters influencing their bioavailability.
We describe herein new developments of 3,4-HP derivatives, as candidates for potential clinical applications, including the results of different drug design strategies, based on the exploitation of the polydenticity and the extra-functionalization of chelating agents to strength the interaction with the metal ions and with specific biological targets; also model studies for combined therapy associated to the use of two structurally different chelators to enhance cell-compartment accessibility. Results of *in vivo* tests, involving a radiotracer (^{67}Ga) in mice models, are also discussed in terms of structure-activity relationship.

ASSESSMENT OF PYRIDOXAL ISONICOTINOYL HYDRAZONE (PIH) AND ITS ANALOGS AS CARDIOPROTECTANTS IN ANTHRACYCLINE-INDUCED CARDIOMYOPATHY.

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Anthracyclines are among the most effective and widely used anticancer drugs. Their usefulness is, however, limited by cumulative dose-dependent cardiomyopathy. Iron-mediated oxidative stress has long been proposed as a pivotal pathophysiological mechanism. To date, dexrazoxane (ICRF-187, a prodrug yielding an EDTA-like metal chelator) is the only cardioprotective agent whose effectiveness has been well documented under both experimental and clinical settings. Since the use of dexrazoxane suffers from certain disadvantages (especially its haematologic adverse effects and high cost), alternative agents with potentially cardioprotective effects are being investigated.
In this study we aimed to assess whether pyridoxal isonicotinoyl hydrazone (PIH) and its two analogs (salicyladehyde isonicotinoyl hydrazone – SIH and pyridoxal 2-chlorbenzoyl hydrazone – o-108) can provide effective protection against daunorubicin (DAU)-induced cardiotoxicity in rabbits.
Chronic DAU treatment (3 mg/kg weekly for 10 weeks) induced mortality (33%), left ventricular (LV) dysfunction, a cardiac troponin T (cTnT) plasma level rise as well as typical morphological LV damage. Co-administrations of PIH (25 mg/kg, i.p.) or SIH hydrochloride (1 mg/kg, i.v.), fully prevented premature deaths and the DAU-induced changes were less pronounced in most functional, biochemical as well as morphological parameters. The best results were achieved with o-108 (10 mg/kg, i.p.). All animals survived without a significant drop in the LV ejection fraction (63.2±0.5% vs. 59.2±1.0%, beginning vs. end, n.s.) and their cardiac contractility (LV dP/dt_{max}) was significantly higher than in the DAU-only group (1131±125 vs. 783±53 kPa/s, $p<0.05$), which well corresponded with lower extent and intensity of myocardial damage as assessed by histology. Surprisingly, although higher doses of the chelators (PIH - 50 mg/kg; SIH – 2.5 mg/kg; o-108 – 25 mg/kg) were well tolerated when administered alone, in a combination with DAU they led to rather paradoxical and mostly negative results regarding both cardioprotection and overall mortality.
In conclusion, we show that shielding of free intracellular iron using aroylhydrazone iron chelators offers a meaningful protection against chronic anthracycline cardiotoxicity. However, as this approach loses its potential with higher (but yet non-toxic) chelator doses, we suggest that iron might play more complex role in the pathogenesis of this toxicity than previously assumed.
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EXPLORING THE MECHANISMS UNDERLYING THE CARDIOPROTECTIVE EFFECTS OF DEFERIPRONE

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More than a dozen scientific studies have been published in the last 2 years reporting on deferiprone's cardioprotection from iron-induced toxicity and/or increased survival in patients with iron overload. Reports have included: reduction of cardiac iron, based on MRI techniques; improved cardiac function and/or decreased prevalence of heart disease in β-Thalassemia patients; reduced prevalence of cardiac-related deaths; reversal of iron-induced heart disease; and reduction of mitochondrial iron; all of which speak to the cardioprotective effects of deferiprone. Such reports have abounded since the original findings of Piga et al (Haematologica 2003) which reported that patients switched to deferiprone and maintained on that treatment for four or more years had five times' lower prevalence of new heart disease than patients who remained on desferrioxamine. There are several potential mechanisms that may contribute to greater cardioprotection of deferiprone compared to desferrioxamine. Some have been explored to a greater extent than others and some, at this point, are speculative. However, all of these factors are worth considering as potential reasons for the reported improvement in heart disease and decreased mortality in deferiprone-treated patients during monotherapy or combination therapy with desferrioxamine.

1. Improved removal of iron from within myocytes due to the favourable partition co-efficient and molecular size of both the chelator and the chelate compared with other chelators at physiologically relevant concentrations (Hershko, Ann NY Acad Sci 2005)
2. Prevention of iron-induced redox cycling within cells (Hider, Blood (Abst) 1999)
3. Reduction of non-transferrin-bound iron levels, minimizing the availability of new iron to enter the myocyte (Pootrakul, Blood 2004)
4. A beneficial effect on the cardiovascular system as reflected by an improved endothelial response factor
5. Reduction in atherosclerosis. (Vercellotti, J Surg Res, 1997)

The data supporting the view that these mechanisms may contribute to deferiprone's cardioprotection in patients with iron overload will be discussed.

INVESTIGATION OF 3-HYDROXYPYRIDIN-4-ONE CONJUGATE AS A NOVEL ORAL IRON CHELATOR.

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Introduction: Secondary iron overload is commonly found in β-thalassaemia patients with multiple blood transfusions. This can cause oxidative tissue damage and organ dysfunction. Desferrioxamine (DFO) and deferiprone (DFP) are nowadays used for iron chelation therapy while deferasirox is in phase III clinical trial. Due to side effects and continuous treatment, cheap oral iron chelators are required. This work was performed to study the iron-chelating activity and cytotoxicity of 3-hydroxypyridin-4-one conjugate (HPO conjugate) in vitro.

Methods: The HPO conjugate was chemically synthesized and purified with silica column chromatography. Electrospray ionization mass spectrometry (EIS-MS) was used to determine its molecular weight (MW). Chemical iron binding of the compound was measured spectrophotometrically, and non-transferrin bound iron (NTBI) was assayed using nitrilotriacetic acid chelation/HPLC technique. Cytotoxic effect on HL-60 cells and peripheral blood mononuclear cells (PBMC) was determined with MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay.

Results: The HPO conjugate (MW = 266) dose dependently bound ferric ion to form a red coloured complex with predominant absorption between 480-490 nm. The binding of iron to the HPO conjugate was dose dependent, complete within 10 minutes, and stable up to 16 hours in MOPS pH 7.0 solution. At least 2.5 μM HPO was able to remove approximately 50% of NTBI content in thalassaemic sera; however, the NTBI chelation was unchanged even increasing the HPO conjugate concentrations (5 μM and 10 μM). HL-60 cells treated with HPO conjugate was more viable than those treated with DFO, and was not different from the DFP-treated cells. Interestingly, the HPO conjugate (3.12-100 μM) was slightly toxic to the HL-60 cells (%cell viability>95%) when compared to DFO and DFP.

Discussion and conclusion: HPO conjugate would be a new oral iron chelator that can remove the serum NTBI effectively. The cytoxicity of the HPO conjugate was less than DFO and comparable to DFP. Iron-chelating capacity of the HPO conjugate needs to be further investigated in iron-loaded cells and thalassaemic mice.

OVER 17 YEARS OF EXPERIENCE WITH DEFERIPRONE IN SWITZERLAND

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There are currently 11 patients (5 female, 6 male thalassaemics) included in a long-term observation study of deferiprone monotherapy or combination therapy (DFO/DFP) at the Berne University Children's Hospital.
All of them had previously been on subcutaneous desferrioxamine for 3-6 nights per week for a duration of 4-10 years before being switched to the oral chelator deferiprone. Several other patients dropped out or were lost to follow-up because of refusal to continue any consistent treatment of their transfusional iron overload (patients number BE 3/12/15/16/17) or due to very low body iron stores and massive reduction of transfusional requirements and chelator dosage (BE 18 and 19, both with HbH-alpha thalassaemia). BE13 had a successful BMT. Three patients died (BE6 due to septicaemia; BE9, over 14 months off chelation and 20 weeks pregnant, of cardiac failure; BE10 of AIDS). In this total of 22 patients, arthralgias and arthritis, predominantly gonarthritis, were observed in 3 male patients. Three patients experienced an episode of reversible, clinically asymptomatic agranulocytosis in the course of a viral URI (2 of them while on IFN and ribavirin). Nausea an GI-discomfort occurred in another 2 patients.
Focusing on the remaining 8 real veterans, born between 1962 and 1982, who were started on deferiprone between 1989 and 1991 (BE 1/2/4/5/7/8/11/14), 4 have been on deferiprone exclusively for the duration of 17 (BE 4/5/11) and 15 years (BE14, after high-dose DFO 1990-91). The other 4, all on DFP since 1989, were started on combined therapy DFO/DFP following their liver iron concentration (LIC) assessments and cardiac MRI in 2001. 7/8 patients showed an overall decrease in their serum ferritin (SF) over the years: mean +/-SD SF value of 3436 +/- 1814 mcg/L in 1989, and 742+/-153 mcg/L ($p= 0.026$) in 2005, excluding BE11 with extremely high SF compared to LIC in 2001, who showed an increase of SF and LIC in 2005 and is to start combined therapy now. Four veterans are negative for hepatitis C, one was successfully treated, 3 continue to be positive. Liver iron concentrations were assessed by SQUID in 1994, 2001 and 2005, and by atomic absorption spectroscopy in liver biopsies in 1997 and 1998. LIC decreased in all but BE11, the mean +/-SD LIC was 14.2 +/-5.6 mg/g d.w. in 1994, and 7.0 +/- 3.7 mg in 2005 (excluding BE11; $p=0.04$).
Heart function and iron overload were assessed in 2001 by H/M SIR and in 2005 by H/M SIR and T2*, and LVEF was measured by MRI and ECHO. Only patient BE2, keener on cars, drugs and nicotine than on DFP, had a T2* value of 9.4 ms (H/M SIR 0.8 / LVEV 55%). Cardiac function was normal in the other patients (T2* >20ms, H/M SIR >1.0, LVEV >60%). In 2005, the mean +/-SD values were 1.5 +/- 0.2 for H/M SIR, for LVEV 68.9 +/-3.9% (by MRI), 64.2 +/-4.3% (by ECHO), and 30.3 +/- 8.7ms for T2*.
Four of the veterans hold a full-time job, and 3 work part-time (one had a baby in 2005). All but one are reasonably compliant and mostly content with their quality of life, and after all these years, they trust in and tolerate deferiprone well enough to turn down the offer of being switched to the newly registered alternative.

EFFECTS OF COMBINED CHELATION THERAPY ON SERUM FERRITIN LEVELS AND T2* VALUES

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Introduction: The benefits of the combined oral and parenteral chelation therapy are widely reported especially focusing on reducing myocardial iron loading. The results of dual therapy need further investigation. Herein, we present the efficacy of combined chelation both on iron and liver iron load.
Patients and methods: Five (3 females) patients with Thalassemia Major from Sparta General Hospital (Greece) Thalassemia Unit were enrolled. Inclusion criteria were MRI T2* values , indicating serious heart and/or liver transfusional hemosiderosis. Patients were undergoing chelation monotherapy with DFO , 30-35 mg/kg,5 days a week, subcutaneously. Combined therapy was started with the same dose of DFO and the addition of DFP at a dose of 70-80 mg/kg daily. Elastomeric infusion pumps were used to achieve better compliance. MRI studies were assessed at the beginning of the study and eighteen months later. Echo – Dopler study was performed in order to evaluate systolic function. A complete blood cell count was performed every week for the first month and at transfusion day for the rest period, while hepatic function was monitored by transaminases levels and coagulation tests.Virological study was performed to exclude the possibility of a chronic hepatitis and viral myocarditis.
Results: Baseline mean ferritin value was 2654,8± 1583,7 μg/L .All patients had ferritin values greater than 1000 μg/L, with the exception of one who was enrolled because of an extremely low liver T2* value. In the eighteen months' follow up , mean ferritin values was 289,4 ± 87,9 μg/L. All patients increased T2* liver values, while 2 patients who were presenting very low T2* myocardial values increased them. Echo –Dopler study confirmed improvement of systolic function. Liver and myocardial T2* values are presented in the following figures , related to the ferritin values .No adverse effects (agranulocytosis, transaminases changes) were reported.
Conclusions: Combined therapy seems to be more effective than monotherapy on reducing iron loading , both from liver and heart. Although ferritin values cannot be an accurate index of tissue loading, it seems to reflect the effects of chelation therapy. Elastomeric infusion pumps help patients to react with a better compliance to therapy.

FERTILITY IN THALASSAEMIA FEMALE PATIENTS: IS THERE SOMETHING NEW?

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Aim:
To analyze the findings regarding fertility in thalassaemic females and to explore the role of chelation therapy in this process.

Materials and methods:
11 homozygous β-thalassaemic female patients, receiving regular transfusions since their early life, underwent a total of 16 natural and In Vitro Fertilization (IVF) procedures in a series of attempts to conceive. They were divided in two groups based on their chelation regimen. Group A: consisted of five (5) patients, mean age 31 years (28-34 years), mean serum ferritin 4.500ng/ml (1.500-7.000ng/ml) who were under deferiprone (Ferriprox) therapy. They carried 6 successful pregnancies to term and gave birth to six healthy babies. 3 of these 5 patients underwent in vitro fertilization due to primary hypogonadism and had successful outcomes upon their first effort. There were no complications during pregnancy. All deliveries were planned cesarean sections. Neonates weighed 2.500-2.900grams. Group B: consisted of six (6) patients, mean age 31 years (18-37 years), mean serum ferritin 1.200ng/ml (500-3.000ng/ml) who were under long-term desferrioxamine (Desferal) monotherapy. They carried 5 successful pregnancies to term and gave birth to five healthy babies. These 6 patients underwent 6 failures to conceive during in vitro fertilization due to primary hypogonadism and two successful ones, while three conceived spontaneously. There were two complications during pregnancy. One developed insulin-dependent diabetes and was under insulin supplementation, while another developed heart failure and received desferrioxamine therapy after the 5^{th} month of pregnancy. All deliveries were planned cesarean sections. Neonates weighed 2.400-2.700grams.

Discussion:
It is noteworthy, that despite a much heavier iron overload due to the lack of compliance to the previously prescribed DFO therapy, patients on deferiprone demonstrated significant ease in conceiving and carried complication-free pregnancies in comparison to the female patients on long term DFO therapy. In light of the small molecular weight of deferiprone and its documented ability to penetrate cells and remove intracellular iron, it may be possible that the same effect is exerted upon the endocrine glands. *Farmaki et al, BJH 2006* and *Christoforides et al, 2006*, have both demonstrated significant improvements in glucose metabolism in thalassaemia patients receiving enhanced chelation that included deferiprone. It may be possible that deferiprone clears pathologic deposits of iron from the endocrine glands and the gonads. More studies must be performed to elucidate the possible beneficial role of deferiprone in clearing iron intracellularly and improving endocrine function.

DEVELOPMENT OF PROTEIN IRON CHELATORS AS PHARMACEUTICAL AGENTS

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Indispensable components of our iron withholding defense system are the powerful iron chelating proteins transferrin (Tf) and lactoferrin (Lf). These 80 kDa glycoproteins each can bind strongly two atoms of iron per molecule. Transferrin is responsible for removing free iron from plasma, lymph and cerebrospinal fluid. Lactoferrin has a similar function in exocrine secretions: milk, tears, tubotympanum and nasal exudate, saliva, bronchial mucus, gastrointestinal fluids, cervical-vaginal mucus and seminal fluid. Additionally, Lf is deposited by néutrophils at sites of infectious and neoplastic cell invasions. The ability of Lf to scavenge iron at the acidic pH values of these sites is an essential feature of innate defense.
During the past decade, Tf and Lf with low iron saturation values have begun to be employed as pharmaceutical agents. Transferrin has been extracted and purified from human plasma; its normal iron saturation value of 20-30% has been reduced to 0.3%. The product especially is useful in patients whose iron saturation has risen because of tnyeloblative conditioning in preparation for stem cell or bone marrow transplant and who thus are highly susceptible to baterial infection. The agent might also be effective in premature infants whose high Tf iron saturation is associated with retinopathy and bronchopulmonary dysplasia.
Recombinant human Lf is produced by molds as well as by cells of selected plants and animals. Bovine Lf extracted from cows' milk also is widely available. The protein can be employed to trap excess iron (1) as a nutraceutical for growth of domestic animals and infants, (2) as a preservative to retard spoilage of food and drink products and of meat, and (3) as a pharmaceutical to enhance wound healing and bone repair, and to suppress infectious and neoplastic cell growth.
A small number of microbial pathogens can use human Tf or Lf as iron carriers. Prior to administration of the proteins, patients should be evaluated for possible carriage of these microorganisms.

IRON OUT-OF-BALANCE: A RISK FACTOR FOR ACUTE AND CHRONIC DISEASE

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A remarkably diverse assemblage of acute and chronic diseases are exacerbated by excessive/misplaced iron in specific tissue sites. Patients with these diseases might appropriately be diagnosed and treated not only by general practitioners but also by specialists in such fields as cardiology, dermatology, endocrinology, gastrointestinal, hematologic, hepatic, infectious, neurologic, obstetric, ophthalmic, oncologic, orthopedic, otologic, pediatric, pulmonary and renal.
Mechanisms of iron-associated diseases have been categorized as follows: (1) iron, by itself, has been observed to initiate the disease; (2) iron can be a cofactor in promoting the disease; (3) iron deposits are observed in disease-associated tissue sites; (4) body iron loading is associated with above-normal incidence of the disease; and (5) maternal antibodies can impair normal fetal iron metabolism.
Several dozen diseases will be reviewed and, based on current evidence, mechanisms will be assigned. Additionally, some anomalies will be discussed. For example, such serious epidemic pathogens as the tubercle, plague and typhoid bacilli require,for optimal growth, iron loaded host macrophages. Thus during the past millenium in the countries of central and northern Europe, hemochromatotic persons (whose macrophages are low in iron because of lack of hepcidin) have apparently had a marked survival advantage.

METALLOTHIONEIN REGULATION OF HYPOXIA-INDUCIBLE FACTOR 1 IN DIABETIC HEARTS

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It is widely accepted that diabetes is the major risk factor for the development of heart failure; however, the pathogenesis of diabetic cardiomyopathy remains unclear. The expression of vascular endothelial growth factor (VEGF) and its receptors has been shown to be down-regulated in the heart tissues of diabetic animals and patients. The inadequate expression of VEGF and its receptor suggests that the angiogenic response is compromised, which in turn further exacerbates the hypoxic conditions and leads to severe damage to the heart. On the other hand, defective glucose uptake has been observed in diabetic hearts, which may be due to either insulin-defect- or insulin-resistance-mediated glucose transport (Glut) dysfunction. Hypoxia-inducible factor (HIF)-1α is a transcriptional activator of VEGF and Glut-1, consisting of a regulatory α and a constitutively-expressed β subunits, and is critical for initiating angiogenic and glycolytic responses to hypoxia. The major event that regulates HIF-1 activity is HIF-1α stabilization. HIF-1a is extremely unstable and is quickly degraded under normoxia conditions, which is mediated by oxygen-dependent prolyl hydroxylases. Another key event is HIF-1 transcriptional activation, which is regulated by asparagine hydroxylase. Many metals have been reported to regulate HIF-1 activity, which include cobalt, cadmium, zinc, and copper. One of the metal handling protein-metallothionein (MT) and its transcription factor-metal-responsive transcription factor-1 (MTF-1) have been shown to contribute to HIF-1 activation. We have been investigating the importance of MT and copper for the adaptation of cells to inadequate angiogenesis in diabetic heart. Here, we show that MT is a mediator of HIF-1 in diabetic heart. HIF-1α is up-regulated only in diabetic MT overexpressing transgenic mouse heart, not in diabetic wild-type heart. High glucose attenuated the HIF-1 dependent transcriptional activity under hypoxia conditions in cardiac H9c2 cells and MT was able to inhibit this attenuation. Further study shows that MT is possible to promote HIF-1α nuclear translocation in H9c2 cells under hypoxic conditions. Taken together, this new findings suggest that strategies to induce metal handling protein-MT could offer promise for therapies targeting toward diabetic cardiomyopathy involving inappropriate expression of HIF-1α protein.

HEMOGLOBIN BASED BLOOD SUBSTITUTES

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With the advent of HIV and new variant CJD concern has grown over the safety of blood transfusions, the transfusion services in many developing countries being in jeopardy. What price then a blood substitute that can be guaranteed to be free from infection, needs no blood group matching, is easy to store and has a long shelf life? Such is the goal for scientists who over the last 20 years have been attempting to design a blood substitute. In fact this task is far too difficult and efforts have been concentrated on one important aspect of the function of blood, namely the transportation of oxygen from the lungs to the tissues. There are a number of strategies to achieve this objective and the one I shall consider here is the design of a hemoglobin (Hb) based oxygen carrier.

How can one obtain sufficient quantities of Hb that are guaranteed free of viruses and other infectious agencies? It is not just the quantity of Hb that can be produced, however, that is of concern, the properties of Hb must also be tuned and it is here that difficulties arise. If placed directly into the blood stream i.e. not inside the red blood cell, Hb exhibits a range of undesirable characteristics. Amongst these are the physical properties of Hb, the tendency to dissociate to dimers, the ability of Hb to react with and remove nitric oxide (NO) from the arterial wall and its quasi-peroxidatic activity that enables it to catalyse powerful oxidation reactions that form highly toxic products. All of these problems would be solved if we could encapsulate Hb in a synthetic blood cell but as yet this is too difficult a problem to solve.

Problems regarding osmotic pressure and the dissociation into dimers can be overcome by chemically coupling together the individual chains and then linking together large numbers of Hb molecules to produce stable aggregates that are not filtered by the kidney. Problems associated with tuning the affinity of Hb for oxygen and the ability to bind NO have been partially solved through a very large programme of studies, involving genetically engineered Hb. In these the design of the protein, and hence its function, has been subtlely altered by site-directed mutagenesis.

A similar approach is at the centre of our attempts aimed at reducing the ability of Hb to promote “oxidative stress”.

COMPARATIVE STUDY OF IRON CHELATORS AS REDUCING AGENTS FOR FERRYL HEME: A MECHANISM TO PROTECT AGAINST OXIDATIVE STRESS WITHOUT IRON CHELATION.

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Iron chelators, such as desferrioxamine, ameliorate oxidative damage in conditions of non-iron overload. The mechanism of this therapeutic action of desferrioxamine is complex. In addition to iron chelation desferrioxamine can also act as a reducing agent to remove cytotoxic ferryl forms of myoglobin and hemoglobin and has recently been found to prevent the formation of a highly cytotoxic derivative of myoglobin, the heme to protein cross-linked form. In this study we have examined the effects of a wide range of iron chelators, including the clinically used hydroxypyridinone deferiprone (L1, CP20), on the stability of ferryl myoglobin and their effect on the formation of heme to protein cross-linking. We show that all hydroxypyridinones, as well as many other iron chelators are efficient reducing agents of ferryl myoglobin. The iron chelators are also effective at preventing the formation of cytotoxic derivatives of myoglobin such as heme to protein cross-linking. These results show that the use of these iron chelators *in vivo* may ameliorate oxidative damage through inhibition of both free iron and by reduction of ferryl heme in respiratory heme proteins thereby inhibiting their peroxidatic activity.
We have studied the mechanism through which reduction of ferryl heme in myoglobin and hemoglobin occurs. Analysis of the kinetics of reduction of the wild type and mutated proteins shows that there are two separate pathways for electron transfer, one involving a specific tyrosine residue.

THE USE OF MRI FOR ASSESSING IRON OVERLOAD AND THE PROGRESS OF IRON CHELATION THERAPY.

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Chronic transfusion therapy remains essential for many hemoglobinopathy patients and produces iron-mediated organ damage if left unchecked. Proper dosing of iron chelation therapy requires knowledge of tissue iron burden. Serum markers such as transferrin saturation, ferritin level, and newer "free" iron metrics such as non-transferrin-bound-iron (NTBI) and labile-plasma-iron (LPI) offer important insights into both stored and labile iron pools, are widely available, and are inexpensive. However, plasma surrogates correlate imperfectly with tissue iron levels and are particularly vulnerable to under-recognition of clinically relevant extrahepatic iron deposition.
Since liver iron concentration (LIC) correlates with total body iron stores, liver biopsy can be used to follow iron balance between transfusion and chelation therapies. Unfortunately, liver biopsy is expensive, painful, and carries a 0.5% complication risk making it poorly suited for frequent assessment. Recently, Magnetic Resonance Imaging (MRI) has emerged as an important tool for noninvasive iron estimation. Although the magnetic relaxation rates are organ specific, empiric LIC calibration curves derived for liver R2 and R2* have proven to have good interstudy and intermachine reproducibility. More importantly, MRI can predict cardiac iron buildup before patients develop cardiomyopathy. MRI evidence of cardiac iron ($T2^* < 20$ ms) is associated with a graded risk of cardiac dysfunction.
This presentation presents a simplified view of the physical principles behind noninvasive iron detection by MRI in order to understand the strengths and limitations of this approach. Prior validation work in liver and heart is reviewed. Requirements for making reliable measurements are discussed as well as common sources of estimation error. Lastly, clinical examples are provided to illustrate how these techniques can be invaluable in managing patients with transfusional iron overload.

RENAL IRON DEPOSITION OCCURS IN CHRONICALLY TRANSFUSED SICKLE CELL DISEASE BUT NOT THALASSEMIA MAJOR

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Introduction: Iron overload of somatic organs, including the liver, heart, spleen, pancreas, and pituitary is a common complication of chronic transfusion therapy in thalassemia major (TM). Extrahepatic iron deposition also occurs in sickle cell disease (SCD) but the incidence appears to be lower than for thalassemia major. Renal tubular abnormalities have been described in both TM and SCD and attributed to oxidative damage from iron overload. We performed a pilot study to assess the prevalence of detectable kidney iron in SCD and TM and its relationship to hepatic and cardiac iron.
Methods: Cardiac, liver and renal T2* was measured in 37 TM patients, 16 SCD patients, and 6 normal volunteers using a single-breathhold multiecho gradient echo sequence. All patients were referred for clinical assessment of cardiac and liver iron. All scans were performed an a 1.5 Tesla General Electric CVi magnet running system 9.1.
Results: Renal R2* in normal volunteers was 21.3 ± 6.8 Hz, yielding an upper limit of normal of 38.7 Hz. In TM patients, renal R2* was 21.5 ± 8.7 Hz, with only 1/37 patients having a T2* outside the normal range. However, SCD patients had a mean renal R2* of 75.8 ± 90.8 [11.9-395] Hz with 11/16 patients having T2* > 38.7 Hz ($p<<0.001$). Increased R2* was limited to the cortex. Renal R2* was not correlated with either liver or cardiac R2*.
Discussion: Renal iron deposition is common in SCD and is not related to the degree of transferrin-bound iron (liver R2*) or non-transferrin bound iron (heart R2*). Instead, increased renal R2* in SCD likely represents hemosiderin deposition in the proximal and distal tubules from chronically elevated intravascular hemoglobin from hemolysis. If so, renal R2* may serve as a predictive marker for kidney disease, pulmonary hypertension, systemic hypertension and diastolic dysfunction in SCD. Further work is necessary to determine the clinical correlates of renal iron deposition, both in the kidney as well as the vascular system.

Iron cardiomyopathy in a patient with Sickle Cell Anemia.

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Introduction: Patients with sickle cell anemia (SCA) develop somatic iron overload through either sporadic or regular blood transfusions performed to ameliorate vasoocclusive complications of their disease. However, prior work from our institution and others has failed to demonstrate MRI evidence of cardiac iron deposition in patients with SCA. Endocrine complications are also relatively rare in transfused SCA patients, suggesting that differences in labile iron processing may protect SCA patients from target organ dysfunction.

Case Presentation: We report documented iron cardiomyopathy in a 22 ½ year woman with SCA. The patient was placed on chronic transfusions in 2003 because of recurrent bouts of acute chest syndrome. The patient was already significantly iron overloaded through prior sporadic transfusions, having a hepatic iron concentration (HIC) of 14 mg/g dry weight and a ferritin level of 4,411. However, she had no cardiac iron deposition (T2* 53 ms) and ejection fraction was normal (58%). Routine deferoxamine (40 mg/kg/day, 5 days per week) therapy was prescribed after 1 year of transfusions. The patient had poor compliance with chelation. Repeat MRI was performed in 4/2006 which demonstrated heavy cardiac iron deposition (T2* 7.8 ms), dilated left ventricle and a left ventricular ejection fraction of 43 %. Her ferritin was value 8983 and her estimated HIC was 54.4 mg/g dry weight.

Discussion: There is mounting evidence that patients with SCA have fewer iron-mediated complications than thalassemia major (TM) patients with comparable total body iron burdens. The mechanism of this protection remains unclear but likely involves interactions between inflammatory cytokines and iron processing. This patient represents the first SCA patient that we have documented an abnormal cardiac T2* out of 72 chronically-transfused SCA patients scanned at Children's Hospital Los Angeles. In contrast, more than 50% of adult patients with TM or Blackfan-Diamond anemia demonstrate detectable cardiac iron burdens. Nonetheless, this case demonstrates that the resistance to cardiac iron deposition observed in SCA can be overcome in selective patients with poor chelation compliance. Long term surveillance must be maintained in adults with SCA to better characterize risk factors for iron cardiomyopathy.

COMPARISON OF THE EFFICACY OF COMBINED CHELATION OF DEFERIPRONE AND DEFEROXAMINE WITH OTHER TREATMENTS ON MYOCARDIAL IRON LOAD IN THALASSAEMIA PATIENTS

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INTRODUCTION AND OBJECTIVES:
Thalassaemia patients under regular transfusion therapy have almost inevitable seconder hemosiderosis; of which cardiac iron overload is the most important factor affecting life expectancy. Combination chelation treatments show great promise, while any chelation treatment seem to be slow or ineffective in reducing cardiac iron, alone. However reduction time is of lifely importance when the patient is heavily loaded with iron.
PATIENTS AND METHODS:
In this observational and case control study which lasted for 2.5 years, 86 thalassaemia patients who had consequtive MRI T2* results of the heart were taken into consideration. After the T2* results, the patients were divided into 3 treatment groups according to the results. In order to direct the most suitable treatment known up to date for their myocardial and hepatic iron status. Group A: Combined desferoxamine and deferiprone, group B: Only deferoxamine group, group C: only deferiprone treatment. Serum ferritin levels were also measured minimum 4 times in a year. Ferritin, MRI T2* results were tested for statistical significance with Pearson test. The effect of treatment groups on T2*, and correlation of ferritin with these groups were assessed with Kruskal Wallis and Wilcoxon Rank test.
RESULTS: Mean ferritin levels showed significant correlation with cardiac T2* values in the beginning and at the end of the study ($p=0.009$ and $p=0.01$). The combined treatment group (Group A) showed significant reduction in myocardial iron load assessed by the T2* measurements ($p<0.05$). Group B and C showed reduction in myocardial iron as well (better in deferiprone group) but without statistical significance.
CONCLUSION:
For patients who have heavy myocardial iron, effective combined chelation schedules should be aimed. Although the group using solely deferipron showed good myocardial iron reduction, this group of patients had nearly normal iron at the beginning of the study. For patients having heavy myocardial iron, selection of one chelator alone should be justified for whether the time needed for the clearance of iron will be as fast as combination treatment or not.

16th International Conference on Chelation (ICOC)

ABSTRACTS OF POSTER PRESENTATIONS

The list is on an alphabetical order of the presenting authors

DEPLETED URANIUM REMOVAL EFFECTS OF CHELATING AGENTS IN COMBINATION WITH BICARBONATE IN RATS

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Bicarbonate can aid in the excretion of metals from the kidney by changing the chemical form of the metals and controlling pH in blood. Accordingly, we examined whether bicarbonate itself helped to remove DU, and whether it could accelerate DU excretion when combined with chelating agents. Experiment I: Male rats were injected intramuscularly with 8 mg/kg DU, and then injected intraperitoneally with doses of 640, 1280, and 1920 μmol/kg bicarbonate for 3 days. The bicarbonate injections were administered 5, 30 or 60 min after the DU injection on the first day. Experiment II: A 4 mg/kg DU intramuscular injection was administered to two groups of rats, then the rats in group 1 were injected inraperitoneally with 1280 μmol/kg bicarbonate 5 min later, and then with the chelating agents (Deferiprone:L1, 4,6-dimethyl-1-hydroxypyrimidin-2(1H)-one:AK-4, catechol-3,6-bis(methyleneiminodiacetic-acid):CBMIDA, and ethane-1-hydroxy-1,1-bisphoshonate: EHBP) 30 min later on the first day. They received the same treatment on each of the following two days. The rats in group 2 were injected with chelating agents 30 min[after the DU injection, and then 1280 μmol/kg bicarbonate 60 min later on the first day, and they received the same treatment on each of the following 2 days. In Experiments I and II, the rats were kept in a metabolic cage so the urine and feces could be collected, and they were killed 6 days later. The DU concentrations in the urine; feces; organs such as the liver, kidney, femur and muscles; and serum biochemical constitutes were measured. Results. Experiment I: Bicarbonate was not effective in increasing DU excretions or decreasing DU concentrations in organs despite the doses and timing of the administration. Experiment II: The increase in DU excretions induced by L1 was promoted by both the pre- and post-injections of bicarbonate, and the decrease in DU concentrations in organs induced by AK-4 was promoted by the post-injection of bicarbonate. No effects were obtained with EHBP. The increase in DU excretion and the decrease in DU concentration in organs induced by CBMIDA were promoted by the post-injection of bicarbonate. The results indicated that the DU removal effects of L1, AK-4, and CBMIDA might be increased by combining these agents with bicarbonate if the correct timing for the administration of the bicarbonate is chosen.

FROM COMBINATION THERAPY OF DESFERRIOXAMINE AND DEFERIPRONE TO ALONE USE OF DEFERIPRONE IN TRANSFUSION-DEPENDENT THALASSEMIC PATIENTS

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Introduction: Combination therapy with desferrioxamine (DFX) and Deferiprone (DFO) in the transfusion-dependent thalassemic patients of southern Taiwan was reported and shown to be active and safe in majority of these patients. But we don't know whether they can maintain the effect after changing from combination to alone use of DFO.

Methods and patients: From March 2004 to July 2006, eighteen Chinese transfusion-dependent thalassemic patients (aged 9 to 34 years, seven females and eleven males) were enrolled with regular combination therapy. DFO at the standard daily dose of 75 mg/kg was given to these patients in whom conventional DFX therapy was used at a daily dose of 40 mg/kg given subcutaneously for 5 to 6 days per week. After ferritin level was less than 1000 mg/l, we tried to change therapy model from combination use of DFX and DFO to alone Use of DFO, and the safety and effectiveness of the alone use of DFO was evaluated.

Results: Mean serum ferritin levels on and after combination therapy with DFX and DFO were 2573.8 ± 857.2 (0 month), 1760.9 ± 787.6 (after 3 month), 1296.9 ± 744.2 (after 6 month) and 1312.7 ± 871.0 (after 12 month). Ten of eighteen patients were changed to receive alone therapy with DFO, the time from combination to alone therapy was 6.7 ± 1.25 month. In the alone therapy group, 7 of 10 were kept on alone therapy and only 2 needed to increase DFO dosage. 3 of 10 were returned to combination therapy. In the alone therapy group, mean serum ferritin levels were 2390.9 ± 989.9 (0 month), 1404.2 ± 763.4 (after 3 month), 805.6 ± 282.2 (after 6 month) and 820.1 ± 282.3 (after 12 month). No significant liver function impairment and absolute neutrophil count change were revealed. The adverse effects were mild and transient.

Discussion and conclusion: The combined therapy with DFX and DFO was showed an effective alternative to conventional DFX therapy in non-responding transfusion-dependent thalassemic patients, and majority of them could be changed to alone use of DFO after 6 months of combination therapy. Longer evaluation for alone use of DFO is in progress.

RESULTS OF LONG TERM COMPARATIVE STUDIES IN THALASSAEMIA PATIENTS TREATED WITH DEFEROXAMINE OR COMBINATION THERAPY WITH DEFERIPRONE (L1). SUGGESTIONS FOR EFFECTIVE CHELATION PROTOCOLS.

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Introduction: A new era began with the introduction of Deferiprone (L1) in the treatment of iron overload in thalassaemia about seven years ago in Cyprus. Before that period Deferoxamine (DF) was the only chelator available and thalassaemia patients experienced variable response as a result of variable compliance, toxicity and therapeutic efficiency. The variable response was reflected in the serum ferritin levels of the patients. More than 50% of the patients in Cyprus are now using L1 mainly in combination with DF.

Patient and Methods: Two groups of thalassaemia patients one receiving DF monotherapy (40-80 mg/kg, 2-6 days per week) since infancy and the other a combination of L1/DF therapy (L1 75-100 mg/kg/day and DF 30-60 mg/kg 1-5 days per week) in the last 2-6 years have been studied and compared before and after the introduction of the combination therapy. The patients on the combination therapy were mainly those not complying or having toxicity with DF. Monitoring of the effects of the chelation therapy in both groups was carried by measuring serum ferritin levels every 3-5 months, over a period of 10 years and MRI T2 and T2* levels were also measured and compared in both groups at the end of the study. Interviews on individual basis were carried out regularly with each patient to assess the compliance with the chelation therapy.

Results and Discussion: There were many changes in serum ferritin levels in both groups of patients and in each patient's case the dose protocol appeared to be the major factor determining the level of the change. In a few cases an increase in serum ferritin was observed mainly in patients receiving low doses and not complying with the recommended chelation protocol. A substantial drop in serum ferritin and maintainance at low levels was observed in patients adhering to the ICOC combination protocol and patients receiving at least 50 mg/kg/day, 5 days per week DF. In both cases cardiac MRI T2* levels was within the normal range (>20 ms) in more than 75% of the patients but liver MRI T2* levels was only normal in 10-20% of the cases. Overall there has been a substantial improvement in serum ferritin levels in many cases treated with the combination therapy by comparison to the DF monotherapy group, where the levels were largely unchanged.

Conclusion: The major determining factor in the rapid clearance of excess iron in thalassaemia patients and the maintainance of normal iron stores is the selection and implementation of effective chelation dose protocols. The ICOC combination L1/DF protocol and DF monotherapy at 50 mg/kg/day, 5 days per week, appear to achieve this goal.

1] Kolnagou A et al Hemoglobin, 2006;30: 219-27.

POTENTIOMETRIC AND PHOTOMETRIC INVESTIGATIONS ON THE INTERACTION OF EU(III) WITH DESFERAL (DFO) AND DEFERIPRONE (L1)

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The present paper deals with the interaction of Eu(III) with the pharmaceutical chelating agents Desferal (DFO) and Deferiprone (L1). Eu(III) is used as homologue for the radionuclides Am(III) and Cm(III) because its presents similar aquatic chemistry, possesses useful spectroscopic properties and is non-radioactive. On the other hand, Desferal (DFO) and Deferiprone (L1) are registered drugs, which are used in the treatment of thalassaemia patients. The aim of the study was speciation and determination of the stability of the formed complexes in order to assess possible applicability of the respective drugs for decorporation of radionuclides from contaminated persons. The experiments were based on potentiomentric titration, UV-Vis spectroscopy and Time Resolved Laser Spectroscopy. According to the experimental results Eu(III) forms complexes with L1 which are hydrolyzed at pH > 5. In contrast, the Eu(III) desferal complex is more stable and does not hydrolyze up to pH 8, indicating on the stability of this complex under physiological conditions. The species formed and the corresponding stability constants determined are:

$\log\beta$ (Eu(III)-L1) = 6.5 ± 0.3

$\log\beta$ (Eu(III)-DFO) = 9.6 ± 0.5

ESTIMATION OF CRITICAL THERAPEUTIC LEVELS OF DEFERIPRONE (L1) IN BLOOD OF THALASSAEMIA PATIENTS FOR MAXIMISING IRON REMOVAL FROM TRANSFERRIN AND MINIMISING IRON DEPOSITION AND TOXICITY IN THE HEART AND OTHER ORGANS

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Introduction: Transferrin iron transport is the major pathway of iron donation to all the cells of the body, which is essential for their growth and development. Similarly, the same pathway is used for the deposition of excess iron in the tissues of patients with iron loading conditions such as idiopathic haemochromatosis and transfusional iron overload in thalassaemia, myelodysplasia and sickle cell disease. Cardiac damage is the most serious toxic side effect of transfusional iron overload and the most common cause of death in thalassaemia major patients. Chelators, which can remove iron from transferrin can increase iron excretion and minimise iron deposition in the heart and other organs. Deferiprone (L1) is the only clinically effective chelating drug capable of mobilising iron from transferrin both in vitro and in vivo. Within this context, transferrin iron removal by (L1) is considered as one of its major properties for preventing cardiac iron load and cardiac damage. The level of L1 required for these effects have not yet been determined.
Patients and Methods: Blood samples were obtained at different time intervals from four thalassaemia patients treated with L1. In each blood sample the concentration of L1 as well as the saturation of transferrin were estimated using HPLC and urea polyacrylamide gel electrophoresis (UPAGE) respectively. Blood samples from normal volunteers as well as from the patients just prior to the administration of L1 were also taken and studied. The transferrin saturation of blood samples from each patient following the administration of L1were compared between them and with blood samples obtained prior to the administration of L1 and with those of normal volunteers.
Results: The blood samples of normal volunteers have shown from the UPAGE studies the presence of the four species of transferrin in blood namely the apotransferrin, the two monoferric transferrins and diferric transferrin. In contrast, in all four thalassaemia patients' samples, transferrin was shown from UPAGE to be fully saturated with iron (diferric) prior to the administration of L1. Monoferric as well as apotransferrin gel bands appeared following the administration of L1 especially when the concentration of L1 in blood was exceeding 0.060 mM (0.060-0.300 mM). The higher the concentration of L1 in blood, especially at peaked levels, the higher were the concentration of apotransferrin and monoferric transferrins but lower was the concentration of diferric transferrin. As the concentration of L1 was reduced during the clearance phase, the saturation of transferrin increased and again the diferric transferrin was reformed.
Discussion and conclusion: In all four patients tranferrin was fully saturated with iron prior to the administration of L1.Selection of effective dose protocols of L1 have been shown to maximise iron removal from transferrin at physiological conditions and minimise iron accumulation in the tissues. The higher the level of L1 in blood, the higher the level of iron removal from tranferrin and higher the prospects of reducing iron deposition by transferrin in the tissues.

NEW HEPCIDIN GENE MUTATION IN THALASSEMIA MAJOR (TM) PATIENT.

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Hepcidin, a 25 amino-acid peptide hormone synthesized in the liver, is the key regulator of iron homeostasis. It appears to communicate body status and demand for erythropoiesis to the intestine and in turn modulate intestinal iron absorbition. Iron overload and inflammation up-regulate hepcidin synthesis, while anaemia and hypoxia suppress hepcidin expression. Thalassaemia Major (TM) is a hereditary haemolytic anaemia requiring long-life blood transfusions treatment with consequent iron overload. β-thalassemia is a disorder in which hepcidin is regulated by opposing influences of ineffective erythropoiesis and concomitant iron overload. In order to get further insights on iron regulation in thalassemias, we screened hepcidin and HFE genes in fourty-three TM regularly transfused patients and sixty control subjects. Blood from TM was taken at least 48 hours after chelation therapy and just before blood transfusion. DNA was prepared from peripheral blood, according to standard protocols. Hepcidin and HFE sequences were amplified with PCR using specific primers and PCR products were sequenced, after purification, in a automatic sequencer. Moreover in all patients serum pro-hepcidin was evaluated by ELISA competitive binding assay (DRG, Germany); iron status was evaluated by serum ferritin (SF), percentage of transferrin saturation (TS) by standard procedures and non transferrin bound iron (NTBI) in serum by HPLC after nitrilotriacetic acid (NTA) chelation. Serum IL-6 as inflammation marker was measured by lateral flow immunoassay (Milenia QuickLine, Germany). Molecular analysis detected an undescribed G→T mutation at position +23 of the 5'-untranslated region in a TM patient; no mutations were found in control subjects. The proband has been regularly transfused since the age of 1 year, receiving 2-3 units of packed red cells and treated with Deferoxamine 40 mg/Kg/day 6 days/week. The patient was a 26-years-old homozygous 39 man with high levels of SF (4346 ng/ml), TS (169%) and NTBI (2.10 μM), while serum pro-hepcidin was 269 ng/ml. HFE analysis revealed a homozygous genotype for H63D mutation. The patient's mother was heterozygous for hepcidin and H63D mutation and showed mild iron overload (SF 500 ng/ml; NTBI 0.22 μM) whereas the father, heterozygous only for H63D had normal iron status. According to recent findings (Bridle et al, 2003) our results indicate that hepcidin mutation in association with H63D synergizes the effect on iron homeostasis and it could be responsible for the development of marked iron overload poorly responsive to chelation therapy in β-thalassemia patients.

NTBI VALUES IN THALASSAEMIA PATIENTS ON COMBINED CHELATION (DFP+ DFO) TREATMENT OR ON MONOTHERAPY (DFP OR DFO)

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Beta-thalassaemia major β-TM) is a hereditary haemolytic anaemia resulting from the reduction or complete absence of □-globin chain synthesis. Iron overload due to blood transfusions, despite intensive chelation therapy, remains the major cause of death in TM. Combined chelation with DFO and DFP could be even more effective than mono-therapy to reduce tissue iron burden and circulating form of labile iron as NTBI. The present study was undertaken to evaluate the effectiveness of chelation therapy in modifying iron status and reducing NTBI, on monotherapy (DFO or DFP) or on combined treatment (DFO and DFP), in transfusion-dependent TM patients. We studied thirty-four transfusion-dependent □-thalassemia major (seventeen females and seventeen males, aged 8 to 41 years) regulary transfused. Twelve have been treated with subcutaneous infusions of DFO over 12 h for 5 d each week, at a mean dose of 50mg/kg for a minimum of ten years; twelve have been treated with DFP at a dose of 75 mg/kg/d for two years; ten patients received combined therapy for two years: DFO (40mg/kg/d) was given with subcutaneous infusions over 12 h on three nights each week, DFP (75mg/kg/d) was given in three divided doses, 1 h before breakfast, lunch and dinner daily. During treatment, safety and effectiveness were determined by detailed clinical and laboratory examination. Serum ferritin level, liver and renal function were controlled monthly. Blood samples from thalassaemia patients were taken at least 48 hours after chelation therapy and just before blood transfusion. NTBI was determined when the study started and re-checked after two years. NTBI was evaluated by a chromatographic method Porter *et al* (1996), after nitrilotriacetic acid (NTA) chelation. There were no significant differences in the initial serum ferritin levels, age and sex distribution within the 3 groups. NTBI was significantly lower in patients on combination DFO+DFP (1.15±1.04 μmol/l) compared to patients on DFP (2.61±1.31 μmol/l) (p=0.038) or DFO alone (2.31±1.05 μmol/l) (p=0.035). Serum ferritin did not show significant variations after two years. NTBI decreased of 31% in DFO (1.59±1.03 □mol/l), of 79% in combined therapy patients (0.24±1.23 μmol/l), whereas remained unchanged (2.73±1.59 μmol/l) in patients on DFP alone. No patient showed any change in physical examination and in laboratory parameters (absolute neutrophil count, serum urea creatinine) or liver function. No side-effects occurred in the three groups. In patient receiving DFO alone, compliance was good (defined as taking 75-90% of the recommended doses); in those who received both DFP alone and DFP plus DFO, the compliance was excellent (>90%). The presence of NTBI in serum could be responsible for potential risk to the heart and to other organs. In our study it has been observed a not significant decrease of SF in all patients and this variation can not be associated with the kind of treatment. On the other hand, NTBI showed a significant fall in the combined treatment at the first evaluation and also after two years compared to DFP or DFO monotherapy: no variation has been observed with DFP treatment, low reduction with DFO treatment (31%) and dramatic decrease of NTBI during combined chelation (79%). DFP plus DFO treatment appears to be more effective in improving iron chelation and maintaining low levels of toxic iron fraction compared to DFO or DFP monotherapy.

ANTIOXIDANT CHARACTERISTICS OF NEOCUPROINE IN SOME MODEL SYSTEMS *IN VITRO*

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Introduction: The copper (I) - specific chelator Neocuproine has been frequently used as an inhibitor of copper-mediated damage in biological systems. Neocuproine inhibited Ehrlich ascites tumor cells growth in monolayer culture. The chelator neocuproine, which effectively binds both iron and copper provides a major protection against hydrogen peroxide-induced cardiac damage and against ischemia/reperfusion-induced arrhythmias. In preliminary evaluations, Neocuproine showed significant chemotherapeutic activity against the P388 murine lymphoma in vivo. The present study was designed to investigate some aspects of the effect of Neocuproine on the oxidative processes in some model systems.
Methods: The capability of Neocuproine to scavenge superoxide radicals (scavenging properties) was studied in a system of xanthine-xanthine oxidase to generate superoxide. The amount of superoxide was measured spectrophotometrically by the NBT-test. The ratio of the absorption at 560 nm for the sample containing Neocuproine and the same absorption for the control in percents is called Spectrophotometric scavenger index (SpSI, %). The antioxidant properties of Neocuproine were investigated by measurement of Fe^{2+} and (Fe^{2+} - EDTA) induced lipid peroxidation in a system with an egg liposomal suspension and in a system of liver supernatant. The ability of Neocuproine to affect Fe^{2+}- and (Fe^{2+}-EDTA) induced lipid peroxidation, and products from that reaction MDA are evaluating by spectrophotometry.
Results and discussion: %). The results show that we have 99, 1 % for the 10^{-4} M Neocuproine, 97% for 10^{-5}, and 98,5 SpSI for 10^{-6} M Neocuproine.
The results of our investigation on Fe^{2+}-induced lipid peroxidation in liposomal suspension shows that Neocuproine in the concentration range of 10^{-4}, 10^{-5}, and 10^{-6} M do not change the lipid peroxidation. In contradiction, after Fe^{2+}-induced lipid peroxidation in liver supernatant Neocuproine decrease the level of MDA for concentrations 10^{-4}, 10^{-5}, and 10^{-6} M.
Conclusion: We concluded that Neocuproine does not show superoxide radical scavenging properties. In applied concentration range Neocuproine affects the (Fe^{2+} - EDTA) – induced lipid peroxidation in liposomal suspension in a dose-depend manner.

EFFECT OF LACTOFERRIN ON A MODEL OF ACUTE MYOCARDIAL INFARCTION IN RATS

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Introduction: Lactoferrin (LA) is an innate iron-binding protein which seems to possess many biological activities. In the present study its potentially favorable effect on a model of isoprenaline acute myocardial infarction in rats was investigated.
Methods: Male Wistar rats were divided into four groups with treatments as follows: *control* (saline 2 ml/kg i.v.); *isoprenaline* (ISO, 100 mg/kg s.c.), *LA* (50 mg/kg i.v.); and *LA + ISO* (pretreated with LA 5 min before ISO, dosages as previous). Haemodynamic and histological variables were measured 24 h following drug(s) administration.
Results: LA significantly increased cardiac index, cardiac power index and stroke volume index and prevented drop in these parameters caused by ISO. Furthermore, LA significantly eliminated the negative effect of ISO on peripheral resistence. On the other hand, LA worsened significantly wet ventricles weight and tended to increase heart rate in combination with ISO more than ISO alone. Marked subendothelial damage of the myocardium, particularly in the apex, was observed both in ISO-treated animals as in LA+ISO group when compared to control or LA animals. Such impairment was characterized by large leucocytic infiltration and necrosis of the myocardium leading frequently as far as to myolysis. In LA+ISO group the occurence of described myocardial injury was significantly less pronounced than in the ISO-group.
Conclusion: Certain protective effects of lactoferrin were documented, although some of its activity may be hindered by non-physiological way of application and incompatibility of human lactoferrin with the rat model.

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IN VIVO STANDARDIZED T2* MAP OF NORMAL HUMAN HEART, TO CORRECT T2* SEGMENTAL ARTEFACTS: A MULTISLICE, MULTIECHO T2* MRI APPROACH IN CARDIAC IRON OVERLOADED PATIENTS

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Introduction: Segment-dependent geometric and susceptibility artefacts affect heart Magnetic Resonance Imaging (MRI) segmental T2* assessment in defining the heterogeneous iron distribution in cardiac overloaded patients Objectives To compensate artefactual variations in MRI myocardial segmental analysis of the left ventricle (LV), we developped a segment-dependent correction procedure of the T2* values.
Methods: MRI was performed in 4 groups of 22 subjects each: healthy subjects (I), thalassemia intermedia patients, without iron overload (II); thalassemia major patients, with mild (III) and heavy (IV) iron overload. Basal, medium and apical views of the LV were obtained and analyzed by a custom-written software. T2* mean values were calculated on each 16-segments standardized heart model. Punctual distribution of T2* over the myocardium was assessed and T2* inhomogeneity maps for the 16 segments were obtained. An artefacts correction map was developed using the mean segmental deviations as correction factors and used to normalize segmental data.
Results: The effect of susceptibility differences induced by cardiac veins, and low-scale variations induced by geometrical artefacts were evident. The correction procedure was validated on group I and II. The T2* segmental correction map was able to compensate middle and low resolution variations in T2*, correcting for cardiac/visceral geometric and susceptibility artefacts. Group IV showed no significant presence of segmental artefacts, group III showed a greater variability respect to normal subjects. The correction map failed to compensate these variations when both additive and percentage-based corrections were applied.
Conclusions: We produced a standardized, 3D, 16-segments map of the circumferential distribution of T2* value artefactual variations in normal subjects and developed a T2* correction map to correct segmental measurements in patients with different levels of myocardial iron burden. Using an optimized MRI acquisition technique joined with an appropriate post processing analysis, the segmental approach is robust and can be effectively used to extend the T2* assessment to the entire heart.

HYDROXI(THIO)PYRIDINONES. NEW CHELATING AGENTS FOR POTENTIAL PHARMACOLOGICAL APPLICATIONS

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The development biomimetic compounds with good affinity for specific metal ions and ability, either as ligands or as complexes to be delivered and recognized by target biomacromolecules, is of great relevance for therapy and diagnostic purposes and has been one of the goals of contemporary medicinal chemistry.
As part of an ongoing project, we have developed a series of new chelators, which metal-binding group (MBG) is based on {O,O} or {S,O} bidentate units, with a carbonyl (or thiocarbonyl) and a α-positioned hydroxyl group [C(O,S)-COH] inserted in a benzenoid heterocyclic structure. In particular, compounds with one 3-hydroxy-4-pyridinone (3,4-HP) or 3-hydroxy-4-pyridine-thione (3,4-HTP) chelating units. well known chelating agents with potential applications in medicinal chemistry. The importance of 3,4-HP chelators is well known for their high affinity towards hard metal ions [Fe(III) Al(III)] and medical applications as decorporating agents in situations of metal accumulation.. The novel thio-MBGs present high affinity for soft-hard metal ions, such as Zn(II), the cofactor components of several metalloenzymes, and Pb(II), a highly toxic metal ion. Besides the MBGs, other functional groups are included aimed at optimizing the interactions between the potential drugs (ligands) and the bio-targets, including neuro-receptor, as well as the lipo-hydrophylic character of the compounds, according to their profiled bioavailability.
Therefore, we present herein a set of results associated to a small selection of (3,4-HP)- and (3,4-THP)-based ligands extrafunctionalized with different groups, including arylpiperazine, aminopropargylic and glycosylic groups, mostly aimed at metal decorporation purposes. These new compounds have been prepared and studied, namely for the assessment of their lipo-hidrophilic character; in some specific cases, their metal-affinity and speciation, as well as their effect on the biodistribution of metal-over-loaded mice was also evaluated. The results are discussed based on comparison of different features associated to donor atoms and extrafunctional groups and in view of the diverse potential pharmacological applications.

CARBON MONOXIDE RELEASING COMPOUNDS FOR THE TREATMENT OF INFLAMMATORY DISEASES

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Introduction: The recent discovery of the biological and physiological activities of carbon monoxide as a mediator in anti-inflammatory processes opens an unique opportunity for the development of new drugs based on molecules with the capacity to release therapeutic doses of CO in the body, in a controlled and efficient fashion. In fact, up to the present most of the studies that have been reported administer carbon monoxide as a gas and therapeutic effects requires attaining CO-hemoglobin levels up to 20% which is neither safe nor practical. Organometallic compounds containing CO appear as the best candidates to achieve controlled CO delivery in vivo, either by molecular decomposition or as a result of metabolic action by enzymes or other species present in inflamed tissues such as Reactive Oxygen Species (ROS). Such molecules are named CORMs for CO Releasing Molecules.
In this communication we will discuss the CO releasing profile of several organoiron compounds covering different oxidation states, different ligands and number of COs per molecule and evaluate the possibility of using them as an alternative to the CO inhalation method.
Methods: To mimic an oxidative stress environment, we used as oxidant agents H_2O_2 and TBHP (tert-butylhydroperoxide) aq. sol. 70%. In both cases, the ratio oxidant/substrate used was 100/1, and the concentration of CORM ranges from 5.9 to 15.0 mM in TBHP, and from 6.3 to 15.2 mM in H_2O_2. The assays were done under nitrogen atmosphere at room temperature and protected from light. The gas liberation was detected and quantitated by Gas Chromatography.
Results: All nine Fe CORMs released CO at least with one of the oxidants used. The release rate and extension was found to be related to the structure of the compound. In some cases, a large amount of CO_2 was also detected, which results from ligand oxidation via a Fenton reaction mechanism.
Discussion and Conclusion: The results show that adequate choice of ligands is a feasible way of controlling the CO releasing activity of organoiron CORMs. Using ROS to selectively promote CO release from the molecule is a feasible way of targeting the CORM to inflamed tissues where such concentrations are high. Thereby CO will act as a signalling agent at the site of inflammation. Moreover, some of the compounds have an anti-oxidant capacity, which should reduce the oxidative damage.

CHIMERIC STATUS OF CHILDREN WITH HAEMOGLOBINOPATHIES TREATED WITH STEM CELL TRANSPLANTATION

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Allogeneic stem cell transplantation (SCT) remains the only curative treatment option for patients with thalassaemia and sickle cell disease. Graft rejection or none engraftment are frequent causes of treatment failure. PCR – STR analysis is a highly informative, fast and simple method for monitoring haemopoietic chimerism post SCT.
From 04/1998 until 12/2005 49 patients were examined (456 suffering from beta-thalassaemia and 3 from beta-thalassaemia / sickle cell anaemia) who were subjected to 51 transplantations. The mean age of patients was 12.4 years (range 1.4-21) and the nean time of surveillance was 24.5 months (range 4-72). Chimerical status was examined in Bone Marrow, Peripheral Blood Samples, and in single BFU-E colonies.
Twenty out of 49 patients had Full Donor Chimera (FDC) at all time points investigated, and they all survive disease free. Three out of 49 patients had FDC one month post – transplant but the following 1, 2 and 3 months converted to Mixed Chimera (MC). Two out of three patients survive with MC without need for transfusions. The third patient six months later had full thalassaemia recurrence, received a second transplant from the same donor and survives without disease. Twenty –five out of 49 patients had MC one month post transplant. In 16 of 25 patients, MC was progressively converted to FCD in a mean time of 6 months (range 2-12). These patients survive with normal haemopoiesis except one who died from graft versus host disease. In 7 of 25 patients MC remains stable, and all of them survive free from transfusions. In 2 of 25 patients MC progressively increased and led to graft failure 8 and 4 months post transplant, respectively. Both patients survive with disease. One patient never engrafted and lives with thalassaemia. In 24 patients chimera was investigated in single BFU-E colonies with similar result to those obtained from peripheral blood nucleated cells analysis.
STR-based chimerism analysis post SCT for haemoglobinopathies is a useful tool for graft function surveillance. Mixed Chimera is common, but, when stable, is often related with normal hemoglobin levels and transfusion independency. Increasing mixed chimerism in the first months post SCT, usually heralds graft rejection and its detection can define a group of patients at high risk of disease recurrence that may profit from preemptive immunotherapy.

IMMUNE AND NEURAL STATUS OF THALASSAEMIC PATIENTS RECEIVING DEFERIPRONE OR COMBINED DEFERIPRONE AND DESFERRIOXAMINE CHELATION TREATMENT

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Background: Deferiprone, a recently adopted therapeutic alternative for iron-overloaded patients, has been associated with immunological abnormalities, however, relevant concerns have been raised against a background of dysfunction already reported in thalassaemic patients attributed to iron overload, chronic immunostimulation due to transfusions, splenectomy and DFO. During chelation therapy with Desferrioxamine several complications have been reported due to pharmacological reactions and high dose toxicity concerning acoustic and visual side-effects as well as peripheral nerve disorders by measuring the nerve conduction velocities.
Aims: The aim of our 2 years study was to investigate the immune and neural status of beta-thalassaemic patients receiving Deferiprone, alone or in combination with Desferrioxamine.
Methods: The study involved 44 patients aged 10 – 30 years, 21 treated with L1 (group A) and 23 with L1 combined with DFO (group B).
Results: Humoral and cell-mediated immunological parameters were evaluated before and following one year of chelation treatment. Mean serum immunoglobulin concentrations did not differ between treatment groups, either before or after treatment, being within reference range compared to controls. Thalassaemics demonstrated increased lymphocyte counts both before and after 2-years-treatment, with B- and T-cell counts not differing between treatment groups. CD4+ and CD8+ counts were increased in thalassaemics due to lymphocytosis, although CD4/CD8 ratio did not significantly differ compared to controls. Patients were negative for ANA, anti-dsDNA and AHA both at baseline and following treatment. Anti-SMA were detected in 3 patients (1 in group A and 2 in group B) before treatment initiation and maintained in high titers through out the study. Anti-LKM appeared in 4 patients following treatment (2 in each group), however, co-existence with anti-SMA was not observed. Finally, anti-R1were observed at the end of treatment period in 5 patients (2 in group A and 3 in group B). Antibody presence was not associated with clinical expression of any relevant autoimmune disease
The neurophysiological control included the performance of brainsteam auditory (BAEP), visual (VEP) and somatosensoey (SEP) -evoked potentials, as well as the control of nerve conduction velocities (NCV).
The results of the evoked potential control showed no pathological auditory potentials in none of our patients neither before, nor after the induction of both alternative therapies. The same satisfactory results were found in the controls of the visual and somatosensory evoked potentials. Concerning the tests of measuring the nerve conduction velocities none of the patients showed pathological rates.
Conclusion: In the context of the advantages of orally received chelation treatment as well as the suggested advantages of combined chelation therapy, including synergistic efficacy and lower dosing with limited toxicity, our 2 years study supports the safety of Deferiprone use with regards to immune and neural function.

EFFECT OF DIABETES ON THE CARDIAC PROTECTION INDUCED BY ISCHEMIA PRECONDITIONING

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Diabetes is a serious public health problem. Improvements in the treatment of noncardiac complications from diabetes have resulted in heart disease as a leading cause of death in diabetic patients. Hyperglycemia, as an independent risk factor, was found to causes cardiac damage, leading to diabetic cardiomyopathy, but several other diabetes-related pathogenic factors including hyperlipidemia and inflammation were also found directly to damage the heart, contributing an important role to the development of diabetic cardiomyopathy. Current consensus is that these pathogenic factors, though damage the heart by different mechanisms, have a uniform mechanism to cause the overproduction of reactive oxygen and nitrogen species, which leads to oxidative cardiac injuries. These injuries include abnormal gene expression, altered signal transduction, cardiac cell deaths and remodeling, and cardiomyopathy. The oxidative damage was also evident by abolishing the preconditioning-mediated cardiac protective mechanism since several important antioxidant systems and signaling pathways are impaired under diabetic conditions. Therefore, this presentation will discuss the impairing roles of diabetes in the precondition cardiac protection observed in the animal studies and clinical observation. In regard to proteins, which act key players in the preconditioning-induced cardiac protection mechanisms, we will be focus on the roles of metallothionein in the cardiac protection induced by preconditioning and diabetes-effect on metallothionein expression.

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