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5th December, 2006

Dear Clinician,

GliSODin has been available in Australia since 1st June this year. It has been my pleasure during this first 6 months to provide technical information, seminars and workshops to represent this unique nutraceutical with the potential to intervene at the most fundamental levels of the disease process.

As we all gain a better understanding of its very real possibilities in both preventive and therapeutic interventions, I am frequently asked for technical references to support its role as a superior clinical intervention tool.

I have put together this collection of Abstracts which provide much-needed background information on the role of Superoxide dismutase in preventing and managing disease and ageing. None of these references relate to studies on GliSODin itself; these can be found on the www.glisodin.org website.

Rather, these studies catalogue the history of SOD as a therapeutic intervention tool, at the same time reinforcing the notion that Oxidative Stress underpins disease. As well, I have included studies which refer to the use of *Orgotein*, the bovine injectable form of SOD, available in Europe and elsewhere from the discovery of SOD in 1969 until BSE removed all bovine products from the market in the 1990s.

Newer research examines the role of various *SOD-mimetic* drugs which serve to confirm that quenching superoxide free radical does indeed alter the course of many diseases for the better. These experimental drugs at this stage have various limitations and are not available clinically.

The *Proof of Concept* studies (Vouldoukis et al.) on GliSODin show that it is capable of upregulating all 3 antioxidant enzymes to levels which at least approximate those generated by *Orgotein* or the *SOD-mimetics.* This provides the Clinician with a revolutionary approach to disease management.

Many research groups the world over are coming to the conclusion that uncontrolled Superoxide anion generated primarily by the mitochondria is the common link between seemingly-unrelated pathophysiological states. For example, several of these groups are forming the conclusion that diabetes and cardiovascular disease are different manifestations of the same underlying biochemical defect. No doubt there are many other similar relationships evolving.

The following pages encompass the 3 broad topics, *General Principles of Superoxide dismutase in disease, Orgotein Therapy* and *SOD-mimetic Therapy*, catalogued under 12 sections.

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What much of the research into Oxidative Stress reveals is that antioxidants are not all alike; those antioxidants like vitamin E and beta-carotene which reside in the membrane target different Reactive Oxygen Species (ROS) from those in the cytoplasm or in the mitochondria. Clearly, we are not dealing with a '*one size fits all'* situation. A clear understanding of the different ROS and the antioxidants most targeted to quench or scavenge each one aids in more effective clinical intervention. Vitamin E therapy has proved most disappointing in addressing endothelial dysfunction; this is most likely due to the fact that Vitamin E acts too far *downstream* to be effective. By contrast, those agents which quench Superoxide early in the free radical generation process (such as GliSODin) have demonstrated significant clinical efficacy.

Superoxide dismutase therapy using GliSODin opens up a whole world of new clinical possibilities, providing Clinicians with a targeted tool which gets much closer to addressing disease at a more fundamental source.

Yours in good health,

Christine Houghton B.Sc.,DC.,Grad.Dip.Hum.Nutr.,Dip.AAM
Clinical Biochemist

1. General principles of free radicals in disease etiology

Int J Tissue React. 1985;7(6):513-9. Links

Pharmacology of free radical scavenging in inflammation.

- **Arrigoni-Martelli E**.

  Current available evidence suggesting a role for oxygen free radicals in inflammatory processes is reviewed. The effects of NSAID as scavengers or inhibitors of superoxide anion generation are described. *The anti-inflammatory effect of the superoxide dismutase drug version named Orgotein* is discussed in relation to its enzymic activity and pharmacokinetics profile.

Prostaglandins. 1982 May;23(5):725-30. Links

Study on the effect of superoxide dismutase on arachidonic acid metabolism.

- **Parente L**.

  The effect of orgotein, the drug version of bovine Cu-Zn superoxide dismutase, on PG production by phagocytosing leukocytes has been investigated. Orgotein inhibited PG formation in a dose/dependent manner. Arachidonic acid was able to reverse this inhibitory effect. *In the light of these results it is suggested that anti-inflammatory properties of orgotein may depend, at least in part, on the inhibition of phospholipase activation.*

Biochem Soc Trans. 2006 Oct;34(Pt 5):965-70. Transactions Links

Superoxide, peroxynitrite and oxidative/nitrative stress in inflammation.

- **Salvemini D**, **Doyle TM**, **Cuzzocrea S**. Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, Saint Louis University School of Medicine, 3635 Vista Avenue, St. Louis, MO 63110-0250, U.S.A.

  A considerable body of evidence suggests that formation of potent reactive oxygen species and resulting oxidative/nitrative stress play a major role in acute and chronic inflammation and pain. Much of the knowledge in this field has been gathered by the use of pharmacological and genetic approaches. In this mini review, we will evaluate recent advances made towards understanding the roles of reactive oxygen species in inflammation, focusing in particular on superoxide and peroxynitrite. Given the limited space to cover this broad topic, here we will refer the reader to comprehensive review articles whenever possible.

Prostaglandins Other Lipid Mediat. 2002 Jan;67(1):13-25. Links

Effects of oxidative stress and antioxidant treatments on eicosanoid synthesis and lipid peroxidation in long term human umbilical vein endothelial cells culture.

- **Estrada-Garcia L**, **Carrera-Rotllan J**, **Puig-Parellada P**. Servei de Neurofisiologia, Hospital Universitari de Tarragona Joan XXIII, Tarragona, Spain.

We analyzed the influence of oxidative stress and agents that modify its effect in human umbilical vein endothelial cell cultures (HUVEC). The parameters analyzed were PGI2, TXA2, PGI2/TXA2 ratio, lipid peroxidation and cell viability. Oxidative stress was induced by H2O2. The agents (treatments) that were tested are: antioxidant enzymes (superoxide dismutase and catalase), oxygen free radical scavenger (vitamin E) and eicosanoids of the series 2 and 3 (Arachidonic acid, Eicosapentanoic acid). In this study we show, in long term endothelial cell cultures, the effects of different levels of oxidative stress alone or in combination with the different treatment agents, over the analyzed parameters. With induced oxidative stress alone the results obtained indicate that it has a harmful effect over cell function and viability, and that this effect is dose and time dependent. In absence of oxidative stress in basal situation, none of the treatments assayed showed significant differences compared to control cultures in the different analyzed parameters. *When oxidative stress increased, antioxidant enzymes reduced cell damage and had a protective function, whereas Eicosapentanoic acid and vitamin E presented a lower level of protection*. No beneficial effect was observed with arachidonic acid treatments. *A significant increase in cell survival was observed in culture cells with oxidative stress when they were treated with antioxidant enzymes.*

Eur J Rheumatol Inflamm. 1981;4(2):173-82. Links

Orgotein--(bovine Cu-Zn superoxide dismutase), an anti-inflammatory protein drug: discovery, toxicology and pharmacology.

- **Huber W**.

Orgotein is the generic name adopted by the USAN Council for drug versions of the Cu-Zn superoxide dismutases. It is obtained from bovine liver by a process sequentially involving heat treatment, enzymatic digestion of other proteins and purification to homogeneity by molecular sieve and ion-exchange chromatography. Orgotein occurs naturally in all mammalian cells, with liver, kidney, and erythrocytes being the richest sources. Prior to employing Orgotein in the clinic, a variety of toxicological and pharmacological investigations in animals have been conducted. The results of these studies are being presented. *They indicate that Orgotein possesses a potent anti-inflammatory activity coupled with a pronounced lack of general pharmacological effects, and that its toxicity is of an extremely low order.* Orgotein, a major topic of this workshop, is the generic name adopted in 1971 by the U.S. Adopted Names Council for drug versions of the Cu-Zn superoxide dismutases (SOD).

Arzneimittelforschung. 1979;29(5):781-5. Links

Additional pharmacological aspects of orgotein, a metalloprotein with superoxide-dismutase activity.

- Borrelli F, Serafini C, Mattalia G, Caprino L.

Orgotein is a copper- and zinc-containing protein with superoxide-dismutase activity which can be isolated from bovine liver and erythrocytes. The effects of this drug on adjuvant -induced arthritis in rats, and particularly on the changes in erythrocytes sedimentation rates and plasma fibrinogen levels induced by this experimental infection, were studied. Orgotein was also assayed on nystatin-induced paw edema, passive cutaneous anaphylaxis and Arthus reaction, in rats. Finally, studies on platelet aggregation and the prostaglandin system were conducted. Given at doses of 2.5 and 5 mg/kg i.p. for 14 days to arthritic rats, orgotein normalized the serum changes, inhibited the foot swelling and improved the performance time on the rotating bar. *The drug reduced, after a single dose, the nystatin-induced edema, whilst it showed no effects on the immunological inflammations, platelet aggregation and prostaglandin system.* The probable mechanism of action is discussed.

Cancer Lett. 2005 Sep 28;227(2):133-9. Epub 2005 Jan 8. ELSEVIER FULL-TEXT ARTICLE Links

Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu-Zn superoxide dismutase activities, total glutathione, nitric oxide, and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer.

- Kaynar H, Meral M, Turhan H, Keles M, Celik G, Akcay F. Department of Chest Diseases, Ataturk University, Medical School, 25240 Erzurum, Turkey.

Lung cancer is a common pathology with high mortality due to late diagnosis. Glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), catalase (CAT), xanthine oxidase (XO), Cu-Zn superoxide dismutase (Cu-Zn SOD) activities, total glutathione (TGSH), nitric oxide (NO*), and malondialdehyde (MDA) levels were investigated in erythrocytes of patients with non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), and healthy control group. We aimed to investigate serum GSH, GSH-dependent enzymes activities (GSH-Px and GST), XO, CAT, Cu-Zn SOD activity, and NO*, and MDA levels in patients with NSCLC and with SCLC and correlate with the cancer stage. Erythrocyte MDA, NO*, TGSH levels and erythrocyte SOD, CAT and XO activities were significantly higher in patients with NSCLC and SCLC than in controls. Slightly increased erythrocyte GSH-Px and GST activities were not significantly different from the controls. Erythrocyte MDA level positively correlated with erythrocyte NO* levels in patients with early stage (I+II) in NSCLC groups. Erythrocyte MDA level positively correlated with erythrocyte XO activity in patients with advanced stage (III+IV) in NSCLC groups. However, no other correlation could be found among the parameters in healthy controls and patients with NSCLC and with SCLC. *Results obtained in this study indicate significant changes in antioxidant defence system in NSCLC and SCLC patients, which may lead to enhanced action of oxygen radical, resulting in lipid peroxidation.*

Free Radic Biol Med. 1998 Sep;25(4-5):392-403. Links

Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide.

- Squadrito GL, Pryor WA. Biodynamics Institute, Louisiana State University, Baton Rouge 70803-1800, USA.

  The roles of superoxide (O2.-), peroxynitrite, and carbon dioxide in the oxidative chemistry of nitric oxide (.NO) are reviewed. The formation of peroxynitrite from .NO and O2.- is controlled by superoxide dismutase (SOD), which can lower the concentration of superoxide ions. The concentration of CO2 in vivo is high (ca. 1 mM), and the rate constant for reaction of CO2 with -OONO is large (pH-independent k = 5.8 x 10(4) M(-l)s(-1)). Consequently, the rate of reaction of peroxynitrite with CO2 is so fast that most commonly used scavengers would need to be present at very high, near toxic levels in order to compete with peroxynitrite for CO2. Therefore, in the presence of physiological levels of bicarbonate, only a limited number of biotargets react directly with peroxynitrite. These include heme-containing proteins such as hemoglobin, peroxidases such as myeloperoxidase, seleno-proteins such as glutathione peroxidase, proteins containing zinc-thiolate centers such as the DNA-binding transcription factors, and the synthetic antioxidant ebselen. The mechanism of the reaction of CO2 with OONO produces metastable nitrating, nitrosating, and oxidizing species as intermediates. An analysis of the lifetimes of the possible intermediates and of the catalysis of peroxynitrite decompositions suggests that the reactive intermediates responsible for reactions with a variety of substrates may be the free radicals .NO2 and CO3.-. Biologically important reactions of these free radicals are, for example, the nitration of tyrosine residues. These nitrations can be pathological, but they also may play a signal transduction role, because nitration of tyrosine can modulate phosphorylation and thus control enzymatic activity. In principle, it might be possible to block the biological effects of peroxynitrite by scavenging the free radicals .NO2 and CO3.-. Because it is difficult to directly scavenge peroxynitrite because of its fast reaction with CO2, scavenging of intermediates from the peroxynitrite/CO2 reaction would provide an additional way of preventing peroxynitrite-mediated cellular effects. *The biological effects of peroxynitrite also can be prevented by limiting the formation of peroxynitrite from .NO by lowering the concentration of O2.- using SOD or SOD mimics. Increased formation of peroxynitrite has been linked to Alzheimer's disease, rheumatoid arthritis, atherosclerosis, lung injury, amyotrophic lateral sclerosis, and other diseases.*

Curr Med Chem. 2004 May;11(9):1147-62. online at ingentaconnect Links

Potential therapeutic effect of antioxidant therapy in shock and inflammation.

- Cuzzocrea S, Thiemermann C, Salvemini D. Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Messina, Italy. salvator@unime.it

  Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants and/or a depletion of antioxidants. *A considerable body of recent evidence suggests that oxidant stress plays a major role in several aspects of acute and chronic inflammation and is the subject of this review.* Immunohistochemical and biochemical evidence demonstrate the significant role of reactive oxygen species (ROS) in acute and chronic inflammation. Initiation

of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na+/K+ ATP-ase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of ROS. *All these toxicities are likely to play a role in the pathophysiology of shock, inflammation and ischemia and reperfusion*. (2) Treatment with either peroxynitrite decomposition catalysts, which selectively inhibit peroxynitrite, or with SODm's, which selectively mimic the catalytic activity of the human superoxide dismutase (SOD) enzymes, have been shown to prevent in vivo the delayed tissue injury and the cellular energetic failure associated with inflammation. ROS (e.g., superoxide, peroxynitrite, hydroxyl radical and hydrogen peroxide) are all potential reactants capable of initiating DNA single strand breakage, with subsequent activation of the nuclear enzyme poly (ADP ribose) synthetase (PARS), leading to eventual severe energy depletion of the cells, and necrotic-type cell death. *Antioxidant treatment inhibits the activation of PARS, and prevents the organ injury associated with acute and chronic inflammation.*

N Engl J Med. 1985 Jan 17;312(3):159-63. Links

Oxygen-derived free radicals in postischemic tissue injury.

- **McCord JM**.

It is now clear that oxygen-derived free radicals play an important part in several models of experimentally induced reperfusion injury. Although there are certainly multiple components to clinical ischemic and reperfusion injury, it appears likely that free-radical production may make a major contribution at certain stages in the progression of the injury. The primary source of superoxide in reperfused reoxygenated tissues appears to be the enzyme xanthine oxidase, released during ischemia by a calcium-triggered proteolytic attack on xanthine dehydrogenase. *Reperfused tissues are protected in a variety of laboratory models by scavengers of superoxide radicals or hydroxyl radicals* or by allopurinol or other inhibitors of xanthine oxidase. *Dysfunction induced by free radicals may thus be a major component of ischemic diseases of the heart, bowel, liver, kidney, and brain.*

(Note: Dr Joe McCord is one of the 2 original researchers to identify the SOD enzyme in 1969)

Free Radic Biol Med. 2003 Aug 1;35(3):213-25. ELSEVIER FULL-TEXT ARTICLE Links

Oxidative and nitrosative events in asthma.

- **Andreadis AA**, **Hazen SL**, **Comhair SA**, **Erzurum SC**. Department of Pulmonary and Critical Care Medicine, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

Asthma affects over 15 million individuals in the United States, with over 1.5 million emergency room visits, 500,000 hospitalizations, and 5500 deaths each year, many of which are children. Airway inflammation is the proximate cause of the recurrent episodes of airflow limitation in asthma. Research applying molecular biology, chemistry, and cell biology to human asthma and model systems of asthma over the last decade has revealed that

numerous biologically active proinflammatory mediators lead to increased production of reactive oxygen species (ROS) and the gaseous molecule nitric oxide (NO). Persistently increased ROS and NO in asthma lead to reactive nitrogen species (RNS) formation and subsequent oxidation and nitration of proteins, which may cause alterations in protein function that are biologically relevant to airway injury/inflammation. Eosinophil peroxidase and myeloperoxidase, leukocyte-derived enzymes, amplify oxidative events and are another enzymatic source of NO-derived oxidants and nitrotyrosine formation in asthma. *Concomitant with increased generation of oxidative and nitrosative molecules in asthma, loss of protective antioxidant defense, specifically superoxide dismutase (SOD), contributes to the overall toxic environment of the asthmatic airway.* This review discusses the rapidly accruing data linking oxidative and nitrosative events as critical participants in the acute and chronic inflammation of asthmatic airways.

Am J Respir Crit Care Med. 2002 Dec 15;166(12 Pt 2):S38-43. Full Text FREE ajrccm.atsjournals.org Links

Oxidative stress in airways: is there a role for extracellular superoxide dismutase?

- **Bowler RP**, **Crapo JD**. National Jewish Medical and Research Center, Denver, Colorado 80206, USA. bowlerr@njc.org

Airways are exposed to high levels of environmental oxidants, yet they also have enriched extracellular antioxidants. *Airways disease such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease have evidence of increased oxidative stress, suggesting that reactive oxygen and nitrogen species may overwhelm antioxidant defenses in airway diseases*. Extracellular superoxide dismutase is abundant in pulmonary tissues and protects the lung from increased oxidative stress; however, its role in asthma and other airway diseases has not been fully elucidated. Proteolytic processing of extracellular superoxide dismutase decreases its affinity for the extracellular matrix and may be a mechanism to regulate its distribution during conditions of inflammation or oxidative stress.

2. Orgotein and SOD in Radiation-induced dysfunction

Eur J Rheumatol Inflamm. 1981;4(2):237-43. Links

[Results of a multicenter orgotein study in radiation induced and interstitial cystitis]

- **Kadrnka F**.

Thirty two patients suffering from radiation or interstitial cystitis were treated according to a method, first described by Marberger, Innsbruck. This method consists of intramural PeroxinormR (orgotein) injections into the bladder wall. The injections were administered during 4 to 6 weekly intervals and were given 1 to 6 times. The dose of injection was between 4 and 6 mg, mostly 12 mg, the total dose administered was between 8 and 72 mg. For the evaluation of the treatment objective and subjective criteria, such as cystoscopic picture, pain

at rest, urgency, pollakisuria, incontinence, as well as bladder capacity and micturition frequency, during day and night, were used. In all patients a definite improvement in the symptoms could be observed. *All the parameters and especially the bladder capacity and micturition frequency showed remarkable results. Orgotein treatment tolerability was found to be in 90% of the cases from very good to good.*

Urol Res. 1978;6(4):255-7. Links

Orgotein (superoxide dismutase): a drug for the amelioration of radiation-induced side effects. A double-blind, placebo-controlled study in patients with bladder tumours.

- **Menander-Huber KB**, **Edsmyr F**, **Huber W**.

Orgotein, the drug version of Cu-Zn superoxide dismutases is a new and safe anti-inflammatory agent. Animal experiments have shown that it does not interfere with the tumourolytic effects of radiation or chemotherapy. A double-blind, placebo-controlled study has demonstrated that orgotein injected after each daily irradiation session can be used safely and effectively to ameliorate or prevent the side effects due to high-energy radiation therapy (8,400 or 6,400 rads) of bladder tumours. *Orgotein significantly reduced the signs and symptoms both in the bladder and the bowel, indicating that it provides a therapeutic regimen for control of these side effects, which to date could only be treated symptomatically.*

Minerva Ginecol. 1989 Aug;41(8):413-5. Links

[Use of orgotein in the treatment of reactive damage to the use of high-energy radiation]

[Article in Italian]

- **Grio R**, **Tamburrano F**, **Tetti M**, **Zaccheo F**, **Cellura A**, **Malara D**, **Marchino L**, **Russo P**.

A study has been carried out on 62 patients submitted to pre- and postoperative X-ray treatment, evaluating tolerance and effectiveness of Orgotein administered i.m. *The therapeutic results obtained may be considered very satisfactory.*

Z Urol Nephrol. 1988 May;81(5):305-8. Links

[Therapy of radiation injuries of the bladder with orgotein (Peroxinom)]

- **Maier U**, **Zechner O**. Urologische Universitatsklinik Wien.

30 patients who developed an irradiation bladder after radiotherapy of carcinoma uteri were treated with submucosal infiltration of orgotein. In this homogeneous series (same cancer, dosage and exposition of radiation, same sex) the success rate of 86% improvement of

subjective symptoms or complete healing was excellent, not to be reached by any other mode of treatment. In one patient an anaphylactic shock was seen. Despite of this event--which happens in great published series in only 1 per mille - *Orgotein infiltration should be the therapy of choice in actinic damaged urinary bladders.*

Int J Radiat Oncol Biol Phys. 2004 Nov 15;60(4):1211-9. ELSEVIER FULL-TEXT ARTICLE Links

Efficacy of orgotein in prevention of late side effects of pelvic irradiation: a randomized study.

- Esco R, Valencia J, Coronel P, Carceller JA, Gimeno M, Bascon N. Department of Radiation Oncology, Hospital Clinico Lozano Blesa, Zaragoza, Spain. resco@salud.aragob.es

PURPOSE: To study whether orgotein is effective in preventing late radiation-induced effects. **METHODS AND MATERIALS**: Patients >18 years old who were diagnosed with rectal cancer, had an indication for pelvic irradiation (RT) after surgery, and complied with the selection criteria were randomly assigned at the end of RT to receive orgotein for 7 weeks or no treatment (control). The Radiation Therapy Oncology Group toxicity scale was used to evaluate the RT-induced side effects for up to 2 years. Interruptions due to toxicity, concomitant medication, and non-RT adverse events were also recorded. **RESULTS**: A total of 100 patients were included, with 50 in each group. The groups were comparable in terms of the demographic and baseline characteristics. The orgotein group had statistically significant less late toxicity than the control group (p = 0.036) and nontreated patients had a 66% greater chance of developing late toxicity at 2 years. Grouping toxicity as nonrelevant (Radiation Therapy Oncology Group Grade 0-1) and relevant (Grade 2 or worse), patients given orgotein had a lower incidence of late relevant toxicity than did controls, with statistical significance reached at all follow-up visits. After 2 years, patients not treated with orgotein had, in general, a 37% greater chance of developing late relevant toxicity; this risk was 26% when referring specifically to GI toxicity. No adverse events attributable to orgotein were recorded at any time during the study. **CONCLUSION**: *Orgotein is a safe treatment that significantly prevents the overall occurrence of late toxicity, with toxicity reduction particularly evident in the lower GI tract.*

Tumori. 2002 Sep-Oct;88(5):385-9. Links

The efficacy of orgotein in the treatment of acute toxicity due to radiotherapy on head and neck tumors.

- Valencia J, Velilla C, Urpegui A, Alvarez I, Llorens MA, Coronel P, Polo S, Bascon N, Esco R. Radiotherapeutic Oncology Unit, Hospital Clinico Universitario Lozano Blesa, San Juan Bosco, Zaragoza, Spain.

AIMS AND BACKGROUND: To assess the efficacy of orgotein in the treatment of acute secondary effects of radiotherapy on head and neck tumors. **MATERIAL AND METHODS:** Data were collected on 41 patients who received radiotherapy for tumors of the head and neck. Radiotherapy was the exclusive treatment in 19.5% of cases, with surgery in 24.4%, chemotherapy in 48.8%, and with both in 7.3%. The toxicity requiring use of orgotein was:

oropharynx mucositis (26.8%), dysphagia (34.2%), or both (39%), in grade 2 or more according to the RTOG scale. Orgotein (8 mg i.m.) was administered every 48 hrs until radiotherapy was finished. RESULTS: The overall response rate was 92.5%; a complete response was obtained in 12 patients (30%) and partial in 25 (62.5%). The reduction in toxicity at the end of radiotherapy was one grade in 18 patients (45%), 2 grades in 16 (40%), 3 in 2 patients (5%), and 4 grades in the only patient with grade 4 acute toxicity. A statistically significant influence was shown in obtaining complete response: laryngeal tumor location (P = 0.037), duration of radiotherapy of more than 53 days (P = 0.002), discontinuation for non-toxic reasons (P = 0.008). **CONCLUSIONS**: *We consider that orgotein is highly effective in dealing with acute secondary effects of radiotherapy on the head and neck area.*

Neoplasma. 2002;49(3):201-8. Links

Aerosol orgotein (Ontosein) for the prevention of radiotherapy-induced adverse effects in head and neck cancer patients: a feasibility study.

- **Escribano A**, **Garcia-Grande A**, **Montanes P**, **Miralles L**, **Garcia A**. Department of Radiation Oncology, La Paz University Hospital, Madrid, 28046 Spain. irabanalr@seorl.org

Orgotein is an anti-inflammatory superoxide dismutase agent successfully used in treating several inflammatory diseases. It is also used in treating radiation-induced adverse effects in difference malignancies, notably breast, lung, bladder, prostate, cervix, and head and neck cancers. It is administered either topically or parenterally. To our knowledge, it has never been used before for prophylaxis of radiation-induced adverse effects or in aerosol form. Here we report on the results from a feasibility study on aerosol orgotein (Ontosein) for prevention of acute and deferred radiation-induced adverse effects in patients treated for head and neck malignancies. *Our results show that aerosol orgotein administered before each radiation therapy session may impart some benefits in both incidence and severity of acute and deferred radiation-induced adverse effects in head and neck cancer patients, when compared with historical controls. In addition, aerosol orgotein administration is easy and convenient for both the patient and the radiotherapist.*

Urol Res. 1978;6(4):255-7. Links

Orgotein (superoxide dismutase): a drug for the amelioration of radiation-induced side effects. A double-blind, placebo-controlled study in patients with bladder tumours.

- **Menander-Huber KB**, **Edsmyr F**, **Huber W**.

Orgotein, the drug version of Cu-Zn superoxide dismutases is a new and safe anti-inflammatory agent. Animal experiments have shown that it does not interfere with the tumourolytic effects of radiation or chemotherapy. A double-blind, placebo-controlled study has demonstrated that orgotein injected after each daily irradiation session can be used safely and effectively to ameliorate or prevent the side effects due to high-energy radiation therapy (8,400 or 6,400 rads) of bladder tumours. *Orgotein significantly reduced the signs and symptoms both in the bladder and the bowel, indicating that it provides a therapeutic*

regimen for control of these side effects, which to date could only be treated symptomatically.

Anticancer Res. 1996 Jul-Aug;16(4A):2025-8. Links

Prevention of radioinduced cystitis by orgotein: a randomized study.

- **Sanchiz F**, **Milla A**, **Artola N**, **Julia JC**, **Moya LM**, **Pedro A**, **Vila A**. Center of Radiotherapy and Oncology of Catalonia, Clinica Platon, Barcelona, Spain.

On the basis of previous experiences indicating that the anti-oxidant agent Cu/Zn superoxide dismutase (SOD) is an effective drug in reducing acute and late radiation-induced tissue injury, in the Center of Radiotherapy and Oncology of Catalonia, Barcelona, Spain in 1990 we implemented a randomized prospective study to analyze the incidence and grade of side effects in a group of bladder cancer patients. After surgery patients were randomly allocated to receive either: Option A: Radiotherapy or Option B: Radiotherapy + SOD 8 mgr/IM/day, after each radiotherapeutic application. Between January 1990 and January 1995 a total of 448 patients were included (226 A/ 222 B). *Apart from cutaneous side effects, a highly significant incidence of radioinduced acute cystitis and rectitis was detected in patients not treated by SOD.* Which was similar to the delayed side effects. From our data we can conclude that *SOD is effective in decreasing acute radioinduced damage, and also in preventing the appearance of more delayed disorders.*

Urologe A. 1984 Jan;23(1):65-7. Links

[Therapy of acute radiation cystitis. A case report]

- **Mayer P**, **Marx FJ**, **Schilling A**.

We report a patient with prostate cancer who suffered from severe radiation cystitis after combined interstitial radiation with 125-Iodine-Seeds and external radiation (2000 rad). *This patient was treated very successfully with intramural injection of Orgotein (Peroxinorm) into the bladder wall. Besides discussion of the aetiology of radiation cystitis we report our first favourable results with local and systemic treatment with Orgotein (Peroxinorm).*

3. Arthritic diseases – Rheumatoid and Osteoarthritis

J Rheumatol Suppl. 1991 Feb;27:134-7. Links

A French controlled multicenter study of intra-articular orgotein versus intraarticular corticosteroids in the treatment of knee osteoarthritis: a one-year followup.

- **Mazieres B**, **Masquelier AM**, **Capron MH**. Department of Rheumatology, Rangueil University Hospital, University Paul Sabatier, Toulouse, France.

The efficacy of injection of orgotein was compared with that of betamethasone over a one-year period in 419 patients with osteoarthritis of the knee. The criteria for efficacy were the number of recurrences, the rate of persistence in the trial and, secondarily, Lequesne index and the visual analogue scale. *Though betamethasone was quicker-acting, the efficacy of orgotein at low doses (4 or 8 mg) was comparable with that of the corticosteroid from Week 4 and up to a year after the beginning of the study, at the cost of a greater number of injections and more numerous local side effects.*

Am J Med. 1989 Sep;87(3):295-300. Links

Intra-articular orgotein in osteoarthritis of the knee: a placebo-controlled efficacy, safety, and dosage comparison.

McIlwain H, **Silverfield JC**, **Cheatum DE**, **Poiley J**, **Taborn J**, **Ignaczak T**, **Multz CV**. DDI Pharmaceuticals, Inc., Mountain View, California 94043.

PURPOSE: Superoxide dismutase (orgotein for injection) has been used in managing osteoarthritis for more than seven years in Europe; however, well-controlled studies to establish an optimum dosage regimen have not been conducted. In this study, three orgotein dose/regimens were compared with placebo in terms of efficacy, safety, and duration of effect in patients with active osteoarthritis of the knee. **PATIENTS AND METHODS**: A total of 139 patients with osteoarthritis of the knee were enrolled in the study. Nonsteroidal anti-inflammatory agents were withdrawn to induce a flare of disease activity. Patients were then randomly assigned to receive one intra-articular injection of either placebo or orgotein (8 mg to 32 mg) each week for three weeks. Both investigators and patients evaluated disease activity and adverse experiences at a series of follow-up visits for three months. **RESULTS**: Orgotein was effective in reducing symptoms of osteoarthritis for up to three months after treatment; 16 mg given twice was the most effective and most best-tolerated regimen. Discomfort at the injection site was drug related, although this effect also occurred occasionally after injection of placebo. **CONCLUSION**: *The long-lasting effects of intra-articular superoxide dismutase contribute to a favorable risk-benefit ratio and support the importance of the free-radical anion, superoxide (O2-), in the biochemical pathology of osteoarthritis.*

Scand J Rheumatol. 1984;13(2):108-12. Links

Clinical comparison of orgotein and methylprednisolone acetate in the treatment of osteoarthrosis of the knee joint.

- **Gammer W**, **Broback LG**.

Thirty-six patients with osteo-arthrosis affecting the knee took part in a randomized double-blind study in which intra-articular injections of an anti-inflammatory agent, orgotein (superoxide-dismutase) 8 or 16 mg, was compared with intra-articular methylprednisolone acetate 40 mg. It was found that orgotein can be used safely and effectively and without serious adverse reactions. All patients experienced beneficial effects, mainly regarding the pain during the treatment period of 6 weeks with one injection every second week. At the 6-month follow-up, patients treated with 8 mg orgotein or 40 mg methylprednisolone acetate had

deteriorated, whereas all the patients treated with 16 mg orgotein maintained the improvement achieved during the treatment. *The results, according to the patients' assessment of pain and to the patients' assessment of the overall result, were shown to be statistically significantly better with 16 mg orgotein than with 40 mg methylprednisolone acetate.*

Z Rheumatol. 1983 Jan-Feb;42(1):21-4. Links

[Treatment of epicondylitis with locally injected orgotein (double blind study)]

- **Muller U**, **Moll G**.

The efficacy of locally injected orgotein in epicondylitis was investigated in a double-blind clinical trial. The results of the previously used method of treatment--injection with procaine hydrochloride--were applied as a comparison. In nearly all cases of acute epicondylitis, improvement was seen after orgotein injections. More than 70% of these patients were symptom-free 6 weeks after beginning therapy, a result significantly superior to that of the compared method. Both regimens produced distinctly less favorable results in chronic epicondylitis. *In acute epicondylitis orgotein may be regarded as an alternative to the more common and potentially risky corticoid therapy.*

Lancet. 1981 May 9;1(8228):1015-7. Links

Intrasynovial orgotein therapy in rheumatoid arthritis.

- **Goebel KM**, **Storck U**, **Neurath F**.

30 patients with active classical rheumatoid arthritis affecting the knee took part in a 12-week double-blind trial in which intra-articular injections of orgotein (4 mg/week for 6 weeks) were compared with intra-articular aspirin 4 mg/week for 6 weeks. After 12 weeks clinical and biochemical assessments showed that orgotein was superior to aspirin. Clinical response was measured in terms of the cumulative rheumatoid activity index (RAI) which was based on scores for morning stiffness, range of flexion, pain and 25-foot (7.5 m) walking time. Treatment with orgotein resulted in significant improvement of the RAI; the improvement correlated with findings on knee-joint scanning which showed reduced mean uptake of 99mTc-pyrophosphate. *After intra-articular orgotein injections, synovial fluid IgM and IgG rheumatoid factor levels fell significantly; so did prostaglandin E2 formation and lactate dehydrogenase activity. The changes in the synovial fluid suggest that the anti-inflammatory properties of orgotein may lie in its effect on proliferating synovia.*

Arzneimittelforschung. 1983;33(8):1199-203. Links

Intra-articular orgotein therapy in osteoarthritis of the knee. A double-blind, placebo-controlled trial.

- **Lund-Olesen K**, **Menander-Huber KB**.

Intra-articular efficacy and long-term safety of orgotein, the drug version of Cu-Zn superoxide dismutase, were explored in a 24-week double-blind, placebo-controlled trial in patients with active osteoarthritis (ARA criteria) of the knee joint. Each patient received twelve intra-articular injections at biweekly intervals of orgotein (2 mg) or placebo dissolved in about 2 ml saline injection, USP. No other antiinflammatory medications were permitted. *By the end of the trial, significant efficacy of orgotein over placebo had been reached in the parameters of pain, functional improvement and evaluation of status both by physician and patient.* With the orgotein dose regimen used, improvement of disease activity versus time progressed with a fairly constant slope. In the placebo group a surprisingly large initial response was observed which lasted as long as 12 weeks in some instances before subsiding.

Int J Oral Maxillofac Surg. 1994 Dec;23(6 Pt 2):428-9. Links

Use of superoxide dismutase (SOD) in patients with temporomandibular joint dysfunction--a preliminary study.

- **Lin Y**, **Pape HD**, **Friedrich R**. Department of Oral and Maxillofacial Surgery, University of Cologne, Germany.

The aim of this preliminary study was to investigate and assess the effect of intra-articular injection of superoxide dismutase (SOD) in patients with temporomandibular joint (TMJ) dysfunction who had not responded to conservative therapy in a first study. Thirty joints in 29 patients were studied. The results showed that intra-articular injection of superoxide dismutase was effective in 25 joints (83%). *It was concluded that intra-articular injection of superoxide dismutase may be an alternative therapy for patients with TMJ dysfunction who fail to respond to conservative treatment.*

Minerva Med. 1986 May 19;77(21):947-51. Links

Evaluation of the efficacy of orgotein in a series of patients with hydrarthrosis of the knee

- **Terlizzi N**, **Bonali C**, **Tamburrino V**, **Numo R**.

A study was carried out to verify the efficacy of intra-articular orgotein in patients with different forms of hydrarthrosis of the knee. *The results confirm the value of orgotein for the local treatment of osteoarthritic joint swellings.* However in the presence of marked exudative synovitis processes (chronic primary polyarthritis), the use of orgotein is of limited value.

Allergol Immunopathol (Madr). 1990 Sep-Oct;18(5):297-9. Links

Hypersensitivity reaction after Orgotein (superoxide dismutase) administration.

- **Corominas M**, **Bas J**, **Romeu A**, **Valls A**, **Massip E**, **Gonzalez L**, **Mestre M**, **Buendia E**. Allergy Unit, Hospital de Bellvitge Princeps d'Espanya, Barcelona, Spain.

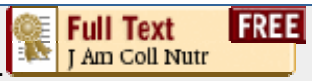
Orgotein is being increasingly used in the treatment of some inflammatory disorders. Up to now no hypersensitivity reaction has been reported. We present the case of an allergic reaction demonstrated by both, "in vivo" and "in vitro" tests. This finding further supports the need for an adequate control during and after orgotein administration.

Handchir Mikrochir Plast Chir. 1984 Mar;16(1):59-63. Links

Intra-articular therapy with superoxide dismutase (orgotein) or cortisone in rheumatoid and arthritic inflammatory finger joint lesions

- **Talke M**.

The use of intra-articular corticosteroid therapy during the acute stage of arthritis is widely accepted. The symmetric involvement in both hands of identical finger joints is the ideal basis for a comparative study of the efficiency of such injections. In a randomized trial corticoid was directly compared with Orgotein, the enzyme "Superoxid-Dismutase" which inactivates oxygen-combinations and thereby interrupts inflammatory reactions. In this way accepted treatment of both sides was possible without the use of a placebo which could be considered as doubtful for ethical reasons. The results were evaluated using the following three reproducible parameters: 1. joint diameter before and after treatment 2. strength of pinch-grip 3. range of motion. Thirty-one patients with ninety-eight affected joints were treated and followed up. Thirty patients showed an improvement upon both treatments. *Twenty-six of the thirty reported identical pain relief for the corticoid and Orgotein treated joint on both sides after approximately three injections per finger joint.* In four cases pain relief in the Orgotein treated joints was less significant than in the corticoid treated ones.

J Am Coll Nutr. 2003 Aug;22(4):311-5. 

Links

Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients.

- **Bae SC**, **Kim SJ**, **Sung MK**. Department of Internal Medicine, Division of Rheumatology, Hanyang University College of Medicine, Seoul, Korea.

OBJECTIVE: *Elevated free radical generation in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA).* The present study was performed to evaluate dietary nutrient intake and plasma oxidant/antioxidant status in RA patients. METHODS: RA patients (n = 97) and their age, gender-matched controls (n = 97)

participated in this cross-sectional case-control study. Nutrient intake was estimated using a semi-quantitative food frequency questionnaire. Twenty subjects from each group provided blood samples, and plasma concentrations of alpha-tocopherol and malondiadehyde (MDA) were measured. Also, plasma activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured. RESULTS: The mean calorie intake of RA patients was lower than that of the healthy controls. Energy-adjusted intake of fat, vitamin A and beta-carotene were significantly lower in patients than those of the control subjects. RA patients had a decreased mean plasma alpha-tocopherol level. *The activity of plasma SOD and GPx in patients was significantly lower than that in control subjects.* **CONCLUSION**: These results suggest proper antioxidant nutrient intake management may reduce free radical generation and improve antioxidant status in RA patients.

Rheumatol Int. 1999;19(1-2):35-7. SpringerLink FULL-TEXT ARTICLE Links

Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis.

- **Ozturk HS**, **Cimen MY**, **Cimen OB**, **Kacmaz M**, **Durak I**.

University of Ankara, Faculty of Medicine, Department of Biochemistry, Turkey.

This study aims to elucidate plasma oxidant/antioxidant status in patients with rheumatoid arthritis (RA). Fasting blood samples were obtained from 24 patients with RA and 20 control subjects. Antioxidant potential (AOP) value, nonenzymatic superoxide radical scavenger activity (NSSA), and malondialdehyde (MDA) levels were measured to establish plasma oxidant/antioxidant status in the patient and control groups. Patients with RA had lower AOP and NSSA but higher MDA levels than those of the control subjects, which was an indication of reduced antioxidant capacity and oxidant stress in these patients. *Results suggest that the antioxidant system is impaired and peroxidation reactions are accelerated in patients with RA. We suppose that therapeutic use of some antioxidants may be beneficial in this regard.*

Clin Chim Acta. 2003 Dec;338(1-2):123-9. ELSEVIER FULL-TEXT ARTICLE Links

Antioxidant status in rheumatoid arthritis and role of antioxidant therapy.

- **Jaswal S**, **Mehta HC**, **Sood AK**, **Kaur J**. Department of Biochemistry, GMCH, H No. 2506-A, Sector 47-C, Chandigarh 160047, India. jasbinderkaur@yahoo.co.in

BACKGROUND: Oxygen free radicals have been implicated as mediators of tissue damage in patients of rheumatoid arthritis (RA*). This study was designed to elucidate plasma oxidant/antioxidant status in rheumatoid arthritis, with the aim of evaluating the importance of antioxidant therapy in the management of this disease.* **METHODS**: The study included 40 patients of rheumatoid arthritis who were randomly divided into two subgroups of 20 each. One group received conventional treatment for 12 weeks and in the other group conventional treatment was supplemented with antioxidants for the same duration. Twenty age- and sex-matched normal individuals constituted the control group.

Blood samples of controls and patients were collected at the time of presentation and analyzed for total thiols, glutathione, vitamin C and malondialdehyde (MDA-marker of oxidative stress). The investigations were repeated in the patients after 12 weeks. RESULTS: The blood concentrations of total thiols, glutathione and vitamin C were found to be significantly lower in rheumatoid arthritis patients as compared to healthy controls, while the concentrations of MDA were much higher. There was a statistically significant increase in the posttreatment concentrations of these antioxidants, along with a decrease in the concentrations of MDA. **CONCLUSIONS**: *The antioxidant defense system is compromised in rheumatoid arthritis patients. There is a shift in the oxidant/antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in the disease. The results suggest the necessity for therapeutic co-administration of antioxidants along with conventional drugs to such patients*. However, due to the limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definite conclusion, in terms of safety and efficacy of adding on antioxidant therapy for the treatment of RA.

Arthritis Rheum. 2005 Dec;52(12):3755-60. Links

Synergistic interaction between methotrexate and a superoxide dismutase mimetic: pharmacologic and potential clinical significance.

- Cuzzocrea S, Mazzon E, di Paola R, Genovese T, Muia C, Caputi AP, Salvemini D.
  University of Messina, Messina, Italy. salvator@unime.it

OBJECTIVE: To investigate the effects of combination therapy with M40403 and methotrexate (MTX) on collagen-induced arthritis (CIA) in rats. **METHODS**: CIA was elicited in Lewis rats that had been assigned to different experimental groups, and the rats were treated daily, starting at the onset of arthritis (day 26), with M40403 2 mg/kg intraperitoneally, MTX 0.15 mg/kg orally, or combination therapy (M40403 2 mg/kg plus MTX 0.015 mg/kg). **RESULTS:** The histopathologic features of CIA in type II collagen-challenged rats included erosion of the articular cartilage and bone resorption. Treatment of rats with MTX 0.15 mg/kg orally delayed the development of clinical signs (days 26-35) and improved histologic status in the knee and paw, as clearly demonstrated by a significant reduction in erosion of the articular cartilage at the joint margins and subchondral bone resorption. Furthermore, radiographic evidence of protection against bone resorption and soft tissue swelling was apparent in the tibiotarsal joints of rats treated with MTX 0.15 mg/kg daily. Furthermore, combination therapy with M40403 2 mg/kg plus MTX 0.015 mg/kg exerted significant protection against the development of arthritis, similar to that observed with MTX alone at a dose of 0.15 mg/kg. In contrast, no significant protection was observed in animals treated with M40403 2 mg/kg alone or with MTX 0.015 mg/kg alone. **CONCLUSION:** *This study provides the first evidence that M40403, a potent superoxide dismutase mimetic, exerts a significant synergistic effect with MTX in rats with CIA.*

4. Orgotein and SOD in Genitourinary Disorders

Urologe A. 1986 Jul;25(4):209-12. Links

[Treatment of contracted bladder with orgotein. Report of experiences]

- **Stroker W**, **Schlutz A**.

On the basis of promising investigations and reports by various authors in the 1970s and early 1980s, since July 1982 *we have been giving a standardized injection of orgotein (see Methods) in the urinary bladder for all forms of contracted bladder that had been unresponsive to previous drug therapy. As the Preparation is well tolerated and highly effective*, *we recommend this method of treatment.* In our experience the only limitation is that orgotein must be injected into the mucous membranes of the bladder under general or regional anesthesia.

Int Urol Nephrol. 1993;25(2):169-72. Links

Orgotein in the treatment of plastic induration of the penis (Peyronie's disease).

- **Primus G**. Department of Urology, LKH, Graz, Austria.

Eighteen patients with Peyronie's disease have been treated with the anti-inflammatory metalloprotein Orgotein, which exhibits a pronounced superoxide dismutase activity. Only patients with severe symptoms were selected. The drug was injected monthly into the indurated areas of the penis. *Marked improvement was noted, especially regarding the loss of pain on erection.* Only one adverse reaction has occurred.

Eur J Rheumatol Inflamm. 1981;4(2):250-9. Links

Orgotein, a new drug for the treatment of Peyronie's disease.

- **Bartsch G**, **Menander-Huber KB**, **Huber W**, **Marberger H**.

Twenty-three patients with Peyronie's disease have been treated with the new anti-inflammatory metalloprotein drug orgotein, which exhibits pronounced superoxide dismutase activity. Administration was done into the indurated areas of the penis by a special syringe. Evaluation of signs and symptoms included measurement of the induration size and consistency as well as the degree of the deviation of the penis. *The patients responded well to orgotein therapy, especially regarding the loss of pain on erection.* On long term results also a diminishment of the induration size and penis deviation on erection was observed; the drug administered intraplaqueally was of outstanding safety.

Eur Urol. 1981;7(6):346-8. Links

Peyronie's disease: experience of local treatment with Orgotein.

- **Gustafson H**, **Johansson B**, **Edsmyr F**.

22 patients with Peyronie's disease have been treated with Orgotein, the new anti-inflammatory metalloprotein which pronounced superoxide dismutase activity patients with long-standing and severe symptoms were selected for the trial. The drug was given monthly by injection into the indurated areas of penis. In 19 patients, where the sexual act could be performed with difficulties or the penile angulation made intercourse impossible, *marked improvement was seen or normal function was restored. No adverse reactions by Orgotein were seen.*

Urol Int. 1991;47(4):236-9. Links

Evaluation of conservative therapeutic approaches to Peyronie's disease (fibrotic induration of the penis).

- **Ludwig G**. Urologische Klinik, Stadtisches Krankenhaus, Frankfurt am Main-Hochst, BRD.

Conservative approaches to the treatment of Peyronie's disease are limited. Irradiation is of no value because the efficacy has not been proven and it may cause tissue damage requiring or hampering subsequent surgery. Vitamin-E therapy is ineffective and is also not recommended. p-Aminobenzoate treatment has to be discontinued because of intolerance in one third of the cases, and the response rate is not superior to that of a placebo. *Local infiltration of the plaques with Orgotein (Peroxinorm) results in a marked improvement in just under 50% of cases and is therefore a justified approach in the early stages of the disease.* It is, however, unclear how great the placebo effect and the spontaneous remission rate are. Surgery suited to the individual case must be considered in patients with severe angulation of the penis rendering introitus impossible, calcium deposits, or worsening of the condition despite injections of Peroxinorm.

5. Orgotein and SOD in Ischaemia-Reperfusion

Rinsho Byori. 1989 Sep;37(9):999-1005. Links

[Possible clinical application of SOD and free radical scavengers]

- **Oyanagui Y**.

Ischemia-reperfusion injury in various organs has been discussed in connection with reactive oxygen species (ROS). Xanthine oxidase (XOD) has been believed to be the source of O2- to produce this injury of dogs and rats but XOD is not detected in hearts of men, pigs or rabbits. This suggests the importance of O2- produced by leukocyte NAD(P)H oxidase. We demonstrated in 1976 that 3 injections of Cu, Zn-SOD (superoxide dismutase) (i.v.)

suppressed rat carrageenan paw edema. McCord succeeded with a single injection in the same model using polyethyleneglycol (PEG-SOD) of long retention time in the blood stream. *Michelson's liposomal SOD had clinical effects on Behcet's disease, Crohn's disease etc.* Stylenemaleimide (SMA)-SOD (Inoue) is now under experimental trial and r*ecombinant human SOD (r-h-SOD) is today in phase II stage. The aim is to prevent myocyte damage or for kidney transplantation.* So-called SOD mimics (Cu-complex etc), antioxidants (synthetic propyl gallate or natural flavonoids or tannins) and hydroxyl radical (.OH) scavengers such as DMTU (dimethylthiourea) are considered as a prototype for clinical application. Fe-chelators also attract attention, because Fe+2 produces the most reactive ROS, .OH radical.

Curr Opin Investig Drugs. 2002 Jun;3(6):886-95. Links

Superoxide, superoxide dismutase and ischemic injury.

- **Salvemini D**, **Cuzzocrea S**. MetaPhore Pharmaceuticals Inc, St Louis, MO 63114, USA. dsalvemini@metaphore.com

Oxidative stress results from an oxidant/antioxidant imbalance: an excess of oxidants relative to the antioxidant capacity*. Recent evidence strongly suggests that oxidant stress plays a major role in several aspects of ischemia and reperfusion.* Immunohistochemical and biochemical evidence demonstrate the significant role of reactive oxygen species, in particular superoxide and its reaction product peroxynitrite, formed by the interaction of superoxide and nitric oxide, in endothelial and tissue injury associated with ischemia and reperfusion. Endothelial cell damage, neutrophil activation and infiltration into tissues, lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane sodium/potassium ATPase activity, inactivation of membrane sodium channels and other oxidative protein modifications contribute to the cytotoxic effect of superoxide and peroxynitrite. In addition, superoxide and peroxynitrite trigger DNA strand breakage, with subsequent activation of the nuclear enzyme poly-ADP ribosyl synthetase, a pathway which contributes to the cellular injury in ischemia and reperfusion. *In vivo, removal of superoxide (and thus of peroxynitrite) by superoxide dismutase mimetics (SODm), which mimic the catalytic activity of the human superoxide dismutase enzymes, prevent the cellular energetic failure and tissue damage associated with ischemia and reperfusion and exert an overall beneficial effect in this situation.* The role(s) of superoxide and the potential utility of SODm will be discussed in this review.

6. Orgotein and SOD in Disease of the nervous system

Int J Clin Pharmacol Res. 1985;5(1):59-62. Links

Intrathecal orgotein.

- **Lund-Olesen K**.

During the period 1971-78, *133 intrathecal injections of orgotein or superoxide dismutase have been given, mostly to patients with multiple sclerosis*. Evaluation of the effect was

deficient but the impression was that of improvement and 45% of the 58 patients with multiple sclerosis requested further intrathecal injections. In one case, intrathecal injections had a good effect against allergic headaches after myelography. *Determination of orgotein in samples of cerebrospinal fluid from 16 patients with multiple sclerosis* gave remarkable results in that the content of orgotein was significantly lower than the level reported by other investigators.

Neurosci Lett. 2003 Jul 31;346(1-2):41-4. ELSEVIER FULL-TEXT ARTICLE Links

Neuroprotection against hypoxia-ischemia in neonatal rat brain by novel superoxide dismutase mimetics.

- **Shimizu K**, **Rajapakse N**, **Horiguchi T**, **Payne RM**, **Busija DW**. Departments of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1083, USA. katsuyoshis@aol.com

We investigated the effects of two new low molecular weight nonpeptidyl *superoxide dismutase (SOD) mimetics* (M40403/M40401; MetaPhore Pharmaceuticals) on infarct volume after hypoxia-ischemia injury (H/I) in immature rats. Animals received vehicle or different doses of M40403 or M40401 i.p. 2 h before exposure to 3 h of 8% hypoxia. The infarct volume of the hemisphere ipsilateral to carotid ligation 24 h later was 73.9+/-8.9% in vehicle animals (n=9), and decreased to 39.7+/-7.2% (P<0.05, n=10) in animals treated with 3 mg/kg M40403 and to 37.2+/-6.4% for animals receiving 3 mg/kg M40401 (P<0.05, n=8). *These data indicate that the SOD mimetics* M40403 and M40401 *have protective effects against hypoxic-ischemic brain injury, and suggest the involvement of superoxide anion in neuronal cell injury during H/I.*

7. Cardiovascular Dysfunction – implications for SOD therapy

FASEB J. 2004 Jan;18(1):94-101. Full Text FREE Faseb J Links

Superoxide: a key player in hypertension.

- **Cuzzocrea S**, **Mazzon E**, **Dugo L**, **Di Paola R**, **Caputi AP**, **Salvemini D**. Institute of Pharmacology, University of Messina, Italy.

Superoxide is increased in the vessel wall of spontaneously hypertensive rats (SHR) where, if "blocked," potentiates endothelium-dependent vasodilation. The purpose of this study was to determine the role of superoxide anion in hypertension and its interaction with nitric oxide (NO). For this purpose we used a low molecular weight synthetic superoxide dismutase mimetic (M40403), known to remove selectively superoxide anion. Baseline mean arterial pressure (MAP) was significantly elevated in the SHR compared with its normal counterpart, Wistar Kyoto (WKY). M40403 at a dose (2 mg x kg(-1) x h(-1)), which had no effect in the WKY, significantly decreased MAP in SHR rats. To determine whether superoxide anion increases MAP by inactivating NO, NO synthesis was blocked with N(G) nitro-arginine methyl ester (L-NAME, 3 mg/kg i.v.), a nonselective nitric oxide synthase inhibitor. L-NAME (3 mg/kg,

i.v) blocked the anti-hypertensive effect of M40403 (2 mg/kg over 30 min). When used at a dose that yielded similar increases in MAP, norepinephrine (2.1 microg/kg) failed to alter the anti-hypertensive effects of M40403 in the SHR. To investigate whether the anti-hypertensive effect of M40403 was associated with an improvement of the alterations in vascular reactivity, a separate group of experiments was carried out ex vivo. Endothelium-dependent vasorelaxation to acetylcholine (10 nM-10 microM), an index of endothelial function, was reduced in aortic rings taken from SHR rats when compared with WKY rats. In vivo treatment with M40403 caused an improvement of the degree of the endothelial dysfunction in SHR rats. Furthermore, immunohistochemical analysis for nitrotyrosine (the product formed from the interaction of nitric oxide with superoxide) revealed a positive staining in aorta from SHR rats. The degree of staining for nitrotyrosine was markedly reduced in tissue sections obtained from SHR rats treated with M40403. Our data suggest that overt production of superoxide in SHR couples with nitric oxide, reducing its function and leading to a loss of blood vessel tone and hypertension. Another important effect appears to be at the level of endothelial cellular integrity, where by interacting with nitric oxide, superoxide anion forms peroxynitrite and subsequent endothelial cell dysfunction. By removing superoxide, M40403 restores blood pressure to near-to-normal values.

Pharmacol Ther. 2001 Feb;89(2):187-206. ELSEVIER FULL-TEXT ARTICLE Links

Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications.

- **Wattanapitayakul SK**, **Bauer JA**. Department of Pharmacology, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, 10110, Bangkok, Thailand.

Despite some recent declines, cardiovascular disease (CVD) remains the major cause of death in the United States and worldwide. Most recent advances in the treatment of CVD states have been produced by inhibition of mechanisms involved in disease progress. Many studies conducted in the last decade have *illustrated increased biological oxidative pathways during CVD in animals and humans.* Thus, increased production of reactive oxygen species may be a unifying mechanism in CVD progression, and *antioxidants may have therapeutic value in this setting.* In this review we address the following questions: Do oxidative mechanisms play a role in CVD? Where do the oxidants come from? What are the relevant oxidative events? What are the therapeutic implications?

Am J Hypertens. 2004 May;17(5 Pt 1):450-6. ELSEVIER FULL-TEXT ARTICLE Links

Overexpression of Cu/Zn-superoxide dismutase and/or catalase in mice inhibits aorta smooth muscle cell proliferation.

- **Shi M**, **Yang H**, **Motley ED**, **Guo Z**. Department of Pathology, Anatomy & Cell Biology, Meharry Medical College, Nashville, TN 37208, USA.

BACKGROUND: *Increasing evidence demonstrates that reactive oxygen species, for example, superoxide (O(2)(-.)) and hydrogen peroxide (H(2)O(2)), promote vascular smooth muscle cell (VSMC) proliferation, and that superoxide dismutase (SOD) and catalase work in concert to scavenge O(2)(-.) and H(2)O(2).* This report examined the

effect of overexpressing Cu/Zn-SOD or catalase on epidermal growth factor (EGF)-induced proliferation and mitogen-activated protein kinase (MAPK) phosphorylation in VSMCs. **METHODS**: The VSMCs were obtained from the aorta of wild-type mice and transgenic mice overexpressing Cu/Zn-SOD and catalase in combination or overexpressing Cu/Zn-SOD or catalase alone. The VSMC proliferation was measured by cell counting and bromodeoxyuridine incorporation assay. The MAPK phosphorylation was determined with Western blotting. **RESULTS**: Treatment of wild-type VSMCs with EGF significantly increased proliferation and phosphorylation of extracellular signal-regulated kinases (ERK1/2) and p38 MAPK. Overexpression of Cu/Zn-SOD or catalase attenuated EGF-induced phosphorylation of ERK1/2 and p38 MAPK and suppressed EGF-induced proliferation in VSMCs. For example, the EGF-induced phosphorylation of ERK1/2 and p38 MAPK and EGF-induced proliferation in VSMCs overexpressing Cu/Zn-SOD or catalase were significantly less than in wild-type VSMCs. Moreover, VSMCs overexpressing Cu/Zn-SOD and catalase in combination showed significantly less proliferation and less phosphorylation of the MAPKs than those overexpressing Cu/Zn-SOD or catalase alone. **CONCLUSIONS:** *Overexpression of Cu/Zn-SOD and catalase in combination is more efficient in inhibiting VSMC proliferation and MAPK phosphorylation than overexpression of Cu/Zn-SOD or catalase alone.*

8. Chemical Toxicity – implications for SOD therapy

Hum Exp Toxicol. 2001 Jan;20(1):34-7. online at ingentaconnect Links

Aspirin impairs antioxidant system and causes peroxidation in human erythrocytes and guinea pig myocardial tissue.

- **Durak I**, **Karaayvaz M**, **Cimen MY**, **Avci A**, **Cimen OB**, **Buyukkocak S**, **Ozturk HS**, **Ozbek H**, **Kacmaz M**. Department of Biochemistry, Medical Faculty, Ankara University, Turkey.

This study aims to investigate possible effects of aspirin treatment on cellular oxidant/antioxidant system. In the first part of the study, 15 guinea pigs were given aspirin at three different doses (2200, 440 and 10 mg/kg/day) for 30 days and five were fed on the same diet without aspirin. After a month, animals were killed and their hearts were removed for use in analyses. *In the other part, after fasting blood samples were obtained from 11 volunteer subjects, they were given aspirin (approximately 10 mg/kg/day) for 30 days* and second blood samples were obtained after 1 month. Five volunteer subjects also participated as placebo control. Oxidant/antioxidant parameters, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), nonenzymatic superoxide scavenger activity (NSSA), susceptibility to oxidation (SO) and antioxidant potential (AOP) values, were assayed in the samples. *Antioxidant system was found to be impaired in the heart tissue from guinea pigs and in the erythrocytes from volunteer subjects.* AOP and NSSA values were lower and MDA higher after aspirin treatment in both heart tissues and erythrocytes. In guinea pig heart tissue, SO was lower, but GSH-Px and CAT were unchanged after aspirin treatment. In human erythrocytes, SO was unchanged, but GSH-Px and CAT activities were increased after aspirin treatment. Changes in guinea pig heart tissues from animals treated with higher aspirin doses were more drastic relative to those of human erythrocytes, but no meaningful differences were observed between analysis parameters of control and lower-dose (10 mg/kg/day) aspirin-treated animals. *Our results suggest that high-dose aspirin exerts significant toxicity to guinea pig myocardium and*

normal dose aspirin may cause peroxidation in the human erythrocytes due to its oxidant potential. We suppose that antioxidant supplementation may be beneficial for the people using aspirin for longer periods in order to prevent peroxidation damages.

Arch Toxicol. 2000 Nov;74(9):533-8. SpringerLink FULL-TEXT ARTICLE Links

The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro).

- **Gultekin F**, **Ozturk M**, **Akdogan M**. Department of Biochemistry and Clinical Biochemistry, Suleyman Demirel University, School of Medicine, Isparta, Turkey. drfatih2000@hotmail.com

Organophosphates are known primarily as neurotoxins. However, reactive oxygen species (ROS) caused by organophosphates may be involved in the toxicity of various pesticides. Therefore, in this study we aimed to examine how an organophosphate insecticide, chlorpyrifos-ethyl (CE) [0,0-diethyl 0 (3,5,6-trichloro-2-pyridyl) phosphorothioate], affects lipid peroxidation and the antioxidant defense system in vitro. For this purpose, four experiments were carried out. In experiment 1, erythrocyte packets obtained from six (three male, three female) volunteers were divided into six portions, and to each was added CE in both a high concentration range (0, 0.4, 2, 10, 50, 100 g/l) and a low concentration range (0, 0.01, 0.1 g/l). Additionally, each concentration group was divided into five tubes, and incubated at +4 degrees C for 0, 30, 60, 120, and 240 min. *After incubation, the levels of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined in the erythrocytes in all tubes*. In experiment 2, to examine the effect of CE (or its main metabolites) on the activity of purified, commercially available enzymes, CE at concentrations of 0. 0.01, 0.1, 0.4, and 10 g/l was incubated with purified SOD, GSH-Px and CAT at the concentrations observed in control group at the 0 CE concentration level in experiment 1 for 1 h at room temperature (25 degrees C). In experiment 3, the xanthine-xanthine oxidase system was used to determine whether the activities of SOD, GSH-Px and CAT were inactivated other than by CE, for example by superoxide radicals inducing lipid peroxidation in erythrocytes. Samples with xanthine and xanthine oxidase were mixed and incubated for 1 h at room temperature (25 degrees C). In experiment 4, to determine whether enzyme activities were still inhibited if lipid peroxidation was prevented by exogenous antioxidants, experiment 1 was repeated with the CE concentrations of 0.01, 0.1, 0.4, and 10 g/l by adding butylated hydroxytoluene and vitamin E to the medium. The MDA levels were determined spectrophotometrically. Enzymatic methods were used for the determination of SOD, GSH-Px, and CAT activities. The Friedman test and Wilcoxon's Signed Ranks test were used to compare paired groups. MDA values and GSH-Px activities increased with increasing CE concentration and incubation period ($P<0.05$), but SOD and CAT activities decreased with increasing CE concentration and incubation period ($P<0.01$). From these results, it can be concluded that in vitro administration of CE resulted in the induction of erythrocyte lipid peroxidation and significant changes in antioxidant enzyme activities, *suggesting that ROS and/or free radicals may be involved in the toxic effects of CE (organic pesticide).*

Hum Exp Toxicol. 2004 Jan;23(1):9-13. Links

The effects of diazinon on lipid peroxidation and antioxidant enzymes in erythrocytes in vitro.

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Diazinon is one of the most widely used organophosphate insecticides (OPI) in agriculture and public health programs. The aim of this study was to investigate how an OPI, diazinon, affects lipid peroxidation (LPO) and the antioxidant defense system in vitro. For this purpose, two experiments were carried out. In experiment 1, *the effects of various concentrations of diazinon on LPO and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in erythrocytes were studied.* Each diazinon concentration was incubated with a previously prepared erythrocyte samples at +4 degrees C for 0, 60 and 180 min. After incubation, the malondialdehyde (MDA) levels and the activities of SOD, GSH-Px and CAT were determined. In experiment 2, in order to determine the direct effect of diazinon on the activities of SOD, GSH-Px and CAT, the erythrocytes were haemolysed and incubated with the various concentrations of diazinon at +4 degrees C for 0, 60 and 180 min. In experiment 1, MDA levels and the activities of SOD and GSH-Px increased with increasing diazinon concentration and incubation period, but CAT activity remained unchanged. In experiment 2, SOD activity was significantly decreased, and GSH-Px activity was significantly increased. From these results, it can be concluded that in vitro administration of diazinon results in the induction of erythrocyte LPO and changes the activities of antioxidant enzymes, *suggesting that reactive oxygen species may be involved in the toxic effects of diazinon.*

Toxicology. 2005 Mar 15;208(2):273-88. Links

Peroxynitrite and drug-dependent toxicity.

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Peroxynitrite is the product of the diffusion-controlled termination reaction between two radicals, nitric oxide and superoxide and is a strong oxidant and nitrating intermediate. Critical biomolecules like proteins, lipids and DNA react with peroxynitrite via direct or radical-mediated mechanisms, resulting in alterations in enzyme activities and signaling pathways. The biological consequences of peroxynitrite-mediated oxidative modifications depend on the levels of oxidant achieved in vivo and its cellular site of production. *High and prolonged fluxes of peroxynitrite that overcome the endogenous antioxidant mechanisms, end up in disruption of cell homeostasis leading to apoptotic or necrotic cell death.* Several drugs used in modern medicine and agriculture can exert their toxic side effects through mechanisms involving the formation of toxic levels of peroxynitrite, via redox cycling, uncoupling of nitric oxide synthase, stimulation of the endogenous formation of nitric oxide and superoxide or lowering of the antioxidant defenses. Experimental evidence point to peroxynitrite participation in the toxicity of doxorubicin, paraquat, acetaminophen and MPTP (N-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine). *The pharmacology against peroxynitrite-mediated toxicity*

could be oriented towards decreasing the levels of the precursor radicals (i.e. using NOS or oxidases inhibitors, SOD mimetics) or reducing the levels of peroxynitrite itself (peroxynitrite scavengers or decomposition catalysts) and serve to attenuate or neutralize drug-dependent toxicity linked to enhanced peroxynitrite formation.

9. Potential for SOD therapy in hyperglycaemia/ diabetes and its complications

Cardiovasc Diabetol. 2005 Apr 29;4(1):5.

Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice.

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Cardiovascular complications, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes. There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia, causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications. Overproduction and/or insufficient removal of these free radicals result in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids. Despite overwhelming evidence on the damaging consequences of oxidative stress and its role in experimental diabetes, large scale clinical trials with classic antioxidants failed to demonstrate any benefit for diabetic patients. As our understanding of the mechanisms of free radical generation evolves, it is becoming clear that rather than merely scavenging reactive radicals, a more comprehensive approach aimed at preventing the generation of these reactive species as well as scavenging may prove more beneficial. Therefore, new strategies with classic as well as new antioxidants should be implemented in the treatment of diabetes.

Diabetes Metab Res Rev. 2006 May-Jun;22(3):198-203.

Constant and intermittent high glucose enhances endothelial cell apoptosis through mitochondrial superoxide overproduction.

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BACKGROUND*: It has been previously shown that hyperglycemia enhances free radical production, inducing oxidative damage, which in its turn activates the death pathways implicated in cell apoptosis and necrosis*. But the possible involvement of this pathway in the hyperglycemia-induced apoptosis of endothelial cells has not yet been reported. **METHODS**: To verify a possible connection between mitochondrial ROS production and apoptosis induced

by both stable and oscillating high glucose, SOD, MnTBAP and TTFA was added to HUVEC cell culture medium. We measured nitrotyrosine and 8OHdG as oxidative stress parameters and Bcl-2 expression and Caspase-3 expression and activity as apoptosis indicators. **RESULTS:** Our results show that hyperglycemia, both stable or oscillating, increases oxidative stress and endothelial cell apoptosis through ROS overproduction at the mitochondrial transport chain level. **CONCLUSION:** *The prevention of mitochondrial oxidative damage seems to be a future important therapeutic strategy in diabetes*.

Diabetes Care. 2003 May;26(5):1589-96. Full Text FREE Diabetes Care Links

New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy.

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Evidence implicates hyperglycemia-derived oxygen free radicals as mediators of diabetic complications. *However, intervention studies with classic antioxidants, such as vitamin E, failed to demonstrate any beneficial effect.* Recent studies demonstrate that a single hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain seems to be the first and key event in the activation of all other pathways involved in the pathogenesis of diabetic complications. These include increased polyol pathway flux, increased advanced glycosylation end product formation, activation of protein kinase C, and increased hexosamine pathway flux. Superoxide overproduction is accompanied by increased nitric oxide generation, due to an endothelial NOS and inducible NOS uncoupled state, a phenomenon favoring the formation of the strong oxidant peroxynitrite, which in turn damages DNA. DNA damage is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP-ribose) polymerase. Poly(ADP-ribose) polymerase activation in turn depletes the intracellular concentration of its substrate NAD(+), slowing the rate of glycolysis, electron transport, and ATP formation, and produces an ADP-ribosylation of the GAPDH. These processes result in acute endothelial dysfunction in diabetic blood vessels that, convincingly, also contributes to the development of diabetic complications. *These new findings may explain why classic antioxidants, such as vitamin E, which work by scavenging already-formed toxic oxidation products, have failed to show beneficial effects on diabetic complications* and may suggest new and attractive "causal" antioxidant therapy. New low-molecular mass compounds that act as SOD or catalase mimetics or L-propionyl-carnitine and lipoic acid, which work as intracellular superoxide scavengers, improving mitochondrial function and reducing DNA damage, may be good candidates for such a strategy, and preliminary studies support this hypothesis. This "causal" therapy would also be associated with other promising tools such as LY 333531, PJ34, and FP15, which block the protein kinase beta isoform, poly(ADP-ribose) polymerase, and peroxynitrite, respectively. *While waiting for these focused tools, we may have other options: thiazolinediones, statins, ACE inhibitors, and angiotensin 1 inhibitors can reduce intracellular oxidative stress generation, and it has been suggested that many of their beneficial effects, even in diabetic patients, are due to this property.*

Curr Vasc Pharmacol. 2006 Jul;4(3):215-27. Links

Role of oxidative stress in development of cardiovascular complications in diabetes mellitus.

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Diabetes represents a serious risk factor for the development of cardiovascular problems such as coronary heart disease, peripheral arterial disease, hypertension, stroke, cardiomyopathy, nephropathy and retinopathy. Identifying the pathogenesis of this increased risk provides a basis for secondary intervention to reduce morbidity and mortality in diabetic patients. Hyperglycemia and protein glycation, increased inflammation, a prothrombotic state and endothelial dysfunction have all been implicated as possible mechanisms for such complications. A linking element between many of these phenomena could possibly be, among other factors, increased production of reactive oxygen species. Vascular endothelial cells have several physiological actions that are essential for the normal function of the cardiovascular system. These include the production of nitric oxide (NO), which regulates vasodilatation, anticoagulation, leukocyte adhesion, smooth muscle proliferation and the antioxidative capacity of endothelial cells. However, under conditions of hyperglycemia, excessive amounts of superoxide radicals are produced inside vascular cells and this can interfere with NO production leading to the possible complications. *This article aims at reviewing the links between reactive oxygen species, diabetes and vascular disease and whether or not antioxidants can alter the course of vascular complications in diabetic patients and animal models. A possible beneficial effect of antioxidants might present a new addition to the range of secondary preventive measures used in diabetic patients.*

In Vitro Cell Dev Biol. 1992 Nov-Dec;28A(11-12):787-90. Links

Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: new insights on the pathophysiological mechanisms of diabetic vascular complications.

- **Curcio F**, **Ceriello A**. Istituto di Patologia Clinica e Sperimentale, Faculty of Medicine, University of Udine, Italy.

Exposure to hyperglycemia slows the rate of proliferation of cultured human endothelial cells. *Recently, it has been reported that glucose may autoxidize generating free radicals* which have been hypothesized to delay cell replication time. To test whether oxidative stress has an effect on delaying cell replication time in hyperglycemic conditions, human endothelial cells cultured from umbilical veins were incubated in 5 or 20 mM glucose, either alone or in the presence of one of three different antioxidants: superoxide dismutase (SOD), catalase and glutathione (GSH). Cells grown in medium with 5 mM glucose, with or without antioxidants, yielded similar population doubling times and cell cycle phase distributions. Significantly lower growth parameters were observed in cells grown in medium with 20 mM glucose, without antioxidants. The presence of the antioxidant reverted them to almost normal growth. *These data show that high glucose levels may delay endothelial cells replication time through the generation of free radicals, suggesting a possible pathophysiological linkage between*

the high levels of glucose and the development of microvascular complications of diabetes, possibly suggesting a new therapeutic approach to prevent such complications.

Curr Diab Rep. 2001 Dec;1(3):282-7. Links

Oxidative stress in diabetic nephropathy: basic and clinical information.

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  Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy, but the intracellular signal transduction pathways regulated by reactive oxygen species (ROS) have not been clearly defined. High glucose (HG) induces intracellular ROS directly via glucose metabolism and auto-oxidation and indirectly through the formation of advanced glycation end products and their receptor binding. ROS mimic the stimulatory effects of HG and upregulate transforming growth factor-beta 1, plasminogen activator inhibitor-1, and extracellular matrix (ECM) proteins by glomerular mesangial cells, thus leading to mesangial expansion. ROS activate other signaling molecules, such as protein kinase C and mitogen-activated protein kinases and transcription factors, such as nuclear factor-kappa B, activator protein-1, and specificity protein 1 leading to transcription of genes encoding cytokines, growth factors, and ECM proteins. Finally, various antioxidants inhibit mesangial cell activation by HG and ameliorate features of diabetic nephropathy. These findings qualify ROS as intracellular messengers and as integral glucose-signaling molecules in glomerular mesangial cells in diabetic nephropathy. *With this new concept, ROS assume a greater importance in the pathogenesis of diabetic nephropathy.* Future studies elucidating other downstream-signaling molecules activated by ROS in mesangial and other renal cells will allow us to understand the final cellular responses to HG, such as proliferation, differentiation, apoptosis, and ECM accumulation. With this new information, we should be able to develop strategies for a more rational treatment of diabetic nephropathy.

Cardiovasc Drug Rev. 2006 Summer;24(2):77-87. Full Text at Blackwell Synergy Links

Oxidative stress as the leading cause of acute myocardial infarction in diabetics.

- **Di Filippo C**, **Cuzzocrea S**, **Rossi F**, **Marfella R**, **D'Amico M**. Department of Experimental Medicine, Second Univeristy of Naples, Naples, Italy.

  The risk factors, such as hypertension and metabolic syndrome, tend to promote heart pathology. These risk factors can aggravate concomitant heart insults as well. Diabetes mellitus represents one of the most important risk factors for the development of heart pathology. By itself it represents a source of vascular and heart dysfunction through formation of reactive oxygen species (ROS) and can compromise the recovery from cardiovascular diseases. This review focuses on the evidence that cellular oxidative stress is the leading cause of the worst outcome of myocardial infarction (MI) in diabetics. Hyperglycemia is viewed in this article as the primary mediator of a cascade of heart damaging events, starting from ROS formation and leading to myocardial ischemia, inflammation and death of myocytes. This article also provides insights into why diverse therapeutic interventions, which have in

common the ability to reduce oxidative stress and inflammation, can impede or delay the onset of complications of myocardial infarction in diabetic patients.

10. Studies using experimental SOD-mimetic drugs

Nat Rev Drug Discov. 2002 May;1(5):367-74. Links

SOD mimetics are coming of age.

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The list of pathophysiological conditions that are associated with the overproduction of superoxide anions expands every day. The most exciting realization is that there seems to be a similarity between the tissue injury that is observed in various disease states, as superoxide anions produce tissue injury and associated inflammation in all tissues in similar ways. Tissue injury and inflammation form the basis of many disease pathologies, including ischaemia and reperfusion injuries, radiation injury, hyperoxic lung damage and atherosclerosis. *This commonality provides a unique opportunity to manipulate numerous disease states with an agent that removes superoxide anions.*

Br J Pharmacol. 2001 Feb;132(4):815-27. Links

Pharmacological manipulation of the inflammatory cascade by the superoxide dismutase mimetic, M40403.

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M40403 is a low molecular weight, *synthetic manganese containing superoxide dismutase mimetic (SODm) that removes superoxide anions* (*O2-) without interfering with other reactive species known to be involved in inflammatory responses (e.g. nitric oxide, NO and peroxynitrite, ONOO-). 2. As such, M40403 represents an important pharmacological tool to dissect the roles of *O2- in acute and chronic inflammation. For this purpose, the pharmacological profile of M40403 was evaluated in carrageenan-induced pleurisy. 3. Injection of carrageenan into the pleural cavity of rats elicited an acute inflammatory response characterized by: fluid accumulation in the pleural cavity which contained a large number of neutrophils (PMNs) as well as an infiltration of PMNs in lung tissues and subsequent lipid peroxidation, and increased production of nitrite/nitrate (NOx), prostaglandin E2 (PGE2), tumour necrosis factor alpha, (TNFalpha), interleukin-1beta (IL-1beta), interleukin-6 (IL-6) and interleukin-10 (IL-10). 4. All parameters of inflammation were attenuated by M40403 except for NOx, PGE2 and IL-10 which remained unaltered. Furthermore, carrageenan induced an upregulation of the adhesion molecules ICAM-1 and P-selectin, as well as nitrotyrosine and poly (ADP-ribose) synthetase (PARS) as determined by immunohistochemical analysis of lung tissues. 5. The degree of staining for the ICAM-1, P-selectin, nitrotyrosine and PARS was reduced by M40403. 6. These results clearly indicate

that *O2- plays a critical role in the development of the inflammatory response by altering key components of the inflammatory cascade. *Therefore, synthetic enzymes of SOD such as M40403, offers a novel therapeutic approach for the management of various inflammatory diseases where these radicals have been postulated to play a role.*

Br J Pharmacol. 2002 Jul;136(6):905-17. nature publishing group Links

Protective effects of M40403, a selective superoxide dismutase mimetic, in myocardial ischaemia and reperfusion injury in vivo.

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Myocardial injury caused by ischaemia and reperfusion comes from multiple pathogenic events, including endothelial damage, neutrophil extravasation into tissue, mast cell activation, and peroxidation of cell membrane lipids. These events are followed by myocardial cell alterations resulting eventually in cell necrosis. An enhanced formation of reactive oxygen species is widely accepted as a stimulus for tissue destruction and cardiac failure. 2. *In this study, we have investigated the cardioprotective effects of the SOD-mimetic M40403 in myocardial ischaemia-reperfusion injury*. M40403 is a low molecular weight, synthetic manganese containing superoxide dismutase mimetic (SODm) that selectively removes superoxide anion. Ischaemia was induced in rat hearts in vivo by ligating the left anterior descending coronary artery. Thirty minutes after the induction of ischaemia, the ligature was removed and reperfusion allowed to occur for at least 60 min. M40403 (0.1-1 mg kg(-1)) was given intravenously 15 min before ischaemia. 3. The results obtained in this study showed that M40403 significantly reduced the extent of myocardial damage, mast cell degranulation and the incidence of ventricular arrhythmias. Furthermore, M40403 significantly attenuated, in a dose-dependent manner, neutrophil infiltration in the myocardium as well as the associated induction of lipid peroxidation. Calcium overload seen post-reperfusion of the ischaemic myocardium was also reduced by M40403. 4. Immunohistochemical analysis for nitrotyrosine revealed a positive staining in cardiac tissue taken after reperfusion: this was attenuated by M40403. Moreover reperfused cardiac tissue sections showed positive staining for P-selectin and for anti-intercellular adhesion molecule (ICAM-1) in the vascular endothelial cells. M40403 treatment markedly reduced the intensity and degree of P-selectin and ICAM-1 in these tissues. No staining for nitrotyrosine, P-selectin or ICAM-1 was found in cardiac tissue taken at the end of the ischaemic period. 5. Overall, M40403 treatment reduced the morphological signs of myocardial cell injury and significantly improved survival. 6. Taken together, these results clearly indicate that M40403 treatment exerts a protective effect against ischaemia-reperfusion-induced myocardial injury, supporting a key role for superoxide anion in reperfusion injuries. *This suggests that synthetic enzymes of SOD such as M40403, offer a novel therapeutic approach for the treatment of ischaemic heart disease where superoxide anion plays a dominant role.*

Hypertension. 2006 Aug;48(2):309-15. Epub 2006 Jun 19.  Links

Oxidative stress mediates the stimulation of sympathetic nerve activity in the phenol renal injury model of hypertension.

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Renal injury caused by the injection of phenol in the lower pole of one kidney increases blood pressure (BP), norepinephrine secretion from the posterior hypothalamic nuclei (PH), and renal sympathetic nerve activity in the rat. Renal denervation prevents these effects of phenol. We have also demonstrated that noradrenergic traffic in the brain is modulated by NO and interleukin-1beta. *In this study, we tested the hypothesis that the increase in sympathetic nervous system (SNS) activity in the phenol renal injury model is because of activation of reactive oxygen species.* To this end, first we examined the abundance of several components of reduced nicotinamide-adenine dinucleotide phosphate oxidase (identified as the major source of reactive oxygen species), including gp91phox/Nox2, p22phox, p47phox, and Nox3 using real-time PCR. Second, we evaluated the effects of 2 superoxide dismutase mimetic, tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl), and superoxide dismutase-polyethylene glycol on central and peripheral SNS activation caused by intrarenal phenol injection. Intrarenal injection of phenol raised BP, NE secretion from the PH, renal sympathetic nerve activity, and the abundance of reduced nicotinamide-adenine dinucleotide phosphate and reduced the abundance of interleukin-1beta and neural-NO synthase mRNA in the PH, paraventricular nuclei, and locus coeruleus compared with control rats. When tempol or superoxide dismutase-polyethylene glycol were infused in the lateral ventricle before phenol, the effects of phenol on BP and SNS activity were abolished. *The studies suggest that central activation of the SNS in the phenol-renal injury model is mediated by increased reactive oxygen species in brain nuclei involved in the noradrenergic control of BP.*

Am J Respir Crit Care Med. 2003 Jun 15;167(12):1600-19.  Links

Superoxide dismutases in the lung and human lung diseases.

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The lungs are directly exposed to higher oxygen concentrations than most other tissues. Increased oxidative stress is a significant part of the pathogenesis of obstructive lung diseases such as asthma and chronic obstructive pulmonary disease, parenchymal lung diseases (e.g., idiopathic pulmonary fibrosis and lung granulomatous diseases), and lung malignancies. Lung tissue is protected against these oxidants by a variety of antioxidant mechanisms among which the superoxide dismutases (SODs) are the only ones converting superoxide radicals to hydrogen peroxide. There are three SODs: cytosolic copper-zinc, mitochondrial manganese, and extracellular SODs. These enzymes have specific distributions and functions. Their importance in protecting lung tissue has been confirmed in transgenic and knockout animal studies. Relatively few studies have been conducted on these enzymes in the normal human lung or in human lung diseases. Most human studies suggest that there is induction of manganese SOD and, possibly, extracellular SOD during inflammatory, but not

fibrotic, phases of parenchymal lung diseases and that both copper-zinc SOD and manganese SOD may be downregulated in asthmatic airways. *Many previous antioxidant therapies have been disappointing, but newly characterized SOD mimetics are being shown to protect against oxidant-related lung disorders in animal models.*

Brain Res. 2003 Feb 14;963(1-2):8-14. ELSEVIER FULL-TEXT ARTICLE Links

Protective effect of a new nonpeptidyl mimetic of SOD, M40401, against focal cerebral ischemia in the rat.

- **Shimizu K**, **Rajapakse N**, **Horiguchi T**, **Payne RM**, **Busija DW**. Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157-1083, USA. katsuyoshis@aol.com

We tested the neuroprotective effects of M40401, a new, low molecular weight (511.4 Da) maganese superoxide dismutase mimetic, against 90 min of middle cerebral artery occlusion (MCAO) in male Wistar rats. Animals received a single injection of vehicle (n=8), 1 mg/kg (n=6), or 3 mg/kg (n=7) 30 min before MCAO. Total lesion volume was reduced only in the group receiving 3 mg/kg M40401 (163.5+/-18.7 versus 43.4+/-7.0 mm(3), for vehicle and M40401, respectively; P<0.05), with almost complete reduction of lesion volume in the cortex but little protection in the basal ganglia. Neurological score was also improved in this group. The dose of 1 mg/kg M40401 had smaller and inconsistent effects on lesion parameters. Administration of a single dose of 3 mg/kg M40401 at 60 min of MCAO or at the end of MCAO (90 min) failed to significantly reduce lesion volume. A single dose of M40401 plus prolonged infusion into the post-MCAO period also failed to decrease lesion volume significantly. *These data indicate that M40401 protects cerebral tissue from ischemic insult when administered before MCAO, probably by limiting damage mediated by detrimental actions of superoxide anion.*

Crit Care Med. 2003 Jan;31(1 Suppl):S29-38. Wolters Kluwer Health | Lippincott Williams & Wilkins Links

Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine.

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Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants, or a depletion of antioxidants*. A considerable body of recent evidence suggests that oxidative stress and exaggerated production of reactive oxygen species play a major role in several aspects of septic shock and ischemia and reperfusion.* Initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na /K adenosine triphosphatase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of reactive oxygen species. In addition, reactive oxygen species are potent triggers of DNA strand breakage, with subsequent activation of the nuclear enzyme poly-adenosine 5'-diphosphate ribosyl synthetase, and eventual severe energy depletion of the cells. Pharmacologic evidence suggests that the peroxynitrite-poly-adenosine

5'-diphosphate ribosyl polymerase pathway contributes to the cellular injury in shock and endothelial injury. Treatment with superoxide dismutase mimetics, which selectively mimic the catalytic activity of the human superoxide dismutase enzymes, has been shown to prevent the cellular energetic failure associated with shock and ischemia-reperfusion and to prevent tissue damage associated with these conditions. In this article, we will briefly review the role of superoxide in septic shock and ischemia-reperfusion injury. *We hope to present evidence to support the potential development of superoxide dismutase mimetics as novel and effective agents in the area of critical care medicine.*

Expert Opin Biol Ther. 2003 Feb;3(1):127-39. Expert Opinion Full text article at www.expertopin.com Links

Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics.

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Evidence of oxidative stress is apparent in both acute and chronic neurodegenerative diseases, such as stroke, Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). Increased generation of reactive oxygen species simply overwhelm endogenous antioxidant defences, leading to subsequent oxidative damage and cell death. Tissue culture and animal models have been developed to mimic some of the biochemical changes and neuropathology found in these diseases. In doing so, it has been experimentally demonstrated that oxidative stress plays a critical role in neuronal cell death. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) have demonstrated therapeutic efficacy in models of neurodegeneration. However, delivery and stability issues have reduced the enthusiasm to clinically develop these proteins. Most recently, SOD mimetics, small molecules which mimic the activity of endogenous superoxide dismutase, have come to the forefront of antioxidant therapeutics. *This review will examine the experimental evidence supporting the use of scavengers of superoxide anions in treating some neurodegenerative diseases, such as stroke, PD and ALS, but also the pitfalls that have met antioxidant molecules in clinical trials.*

Neurosci Lett. 2001 May 25;304(3):157-60. ELSEVIER FULL-TEXT ARTICLE Links

Synthetic superoxide dismutase/catalase mimetics reduce oxidative stress and prolong survival in a mouse amyotrophic lateral sclerosis model.

- **Jung C**, **Rong Y**, **Doctrow S**, **Baudry M**, **Malfroy B**, **Xu Z**. Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 55 Lake Ave North, Worcester, MA 01655, USA.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes motoneuron degeneration, paralysis and death. Mutations in Cu, Zn superoxide dismutase (SOD1) are one cause of this disease. It is widely suspected that increased reactive oxidative species (ROS) is involved in motoneuron degeneration but whether such an involvement plays a role in ALS progression in vivo is uncertain. We treated mice expressing human mutant

SOD1 G93A with EUK-8 and EUK-134, two synthetic SOD/catalase mimetics that have shown efficacy in several animal models of human diseases. *These treatments reduced levels of oxidative stress and prolonged survival. The results suggest that oxidative stress plays an active role in ALS and illustrate the potential for treatment strategies aimed specifically against ROS.*

J Biol Chem. 2005 Aug 12;280(32):29194-8. Epub 2005 Jun 9. Final Version FREE J Biol Chem Links

Superoxide dismutase/catalase mimetics are neuroprotective against selective paraquat-mediated dopaminergic neuron death in the substantial nigra: implications for Parkinson disease.

- **Peng J**, **Stevenson FF**, **Doctrow SR**, **Andersen JK**. Buck Institute for Age Research, Novato, California 94945 and Eukarion, Inc., Bedford, MA 01730, USA.

Exposure of mice to the herbicide paraquat has been demonstrated to result in the selective loss of dopaminergic neurons of the substantia nigra, pars compacta (SNpc) akin to what is observed in Parkinson disease (PD). In this study, we investigate the efficacy of two synthetic superoxide dismutase/catalase mimetics (EUK-134 and EUK-189) in protecting against paraquat-induced dopaminergic cell death in both the rat dopaminergic cell line 1RB3AN27 (N27) and primary mesencephalic cultures in vitro and in adult mice in vivo. Our data demonstrate that pretreatment with either EUK-134 or EUK-189 significantly attenuates paraquat-induced neurotoxicity in vitro in a concentration-dependent manner. Furthermore, systemic administration of EUK-189 decreases paraquat-mediated SNpc dopaminergic neuronal cell death in vivo. *These findings support a role for oxidative stress in paraquat-induced neurotoxicity and suggest novel therapeutic approaches for neurodegenerative disorders associated with oxidative stress such as PD.*

J Neurochem. 2002 Nov;83(4):984-91. Links

Preservation of extracellular glutathione by an astrocyte derived factor with properties comparable to extracellular superoxide dismutase.

- **Stewart VC**, **Stone R**, **Gegg ME**, **Sharpe MA**, **Hurst RD**, **Clark JB**, **Heales SJ**. Department of Molecular Pathogenesis, Division of Neurochemistry, UCL, Institute of Neurology, London, UK.

Cultured rat and human astrocytes and rat neurones were shown to release reduced glutathione (GSH). In addition, GSH oxidation was retarded by the concomitant release of a factor from the cells. One possibility is that this factor is extracellular superoxide dismutase (SOD). In support of this, the factor was found to bind heparin, have a molecular mass estimated to be between 50 and 100 kDa, and CuZn-type SOD protein and cyanide sensitive enzyme activity were demonstrated in the cell-conditioned medium. In addition, supplementation of native medium with exogenous CuZn-type SOD suppressed GSH oxidation. We propose that preservation of released GSH is essential to allow for maximal up-regulation of GSH metabolism in neurones. *Furthermore, cytokine stimulation of astrocytes increased release of the extracellular SOD, and enhanced stability of GSH. This may be a protective*

strategy occurring in vivo under conditions of oxidative stress, and suggests that SOD mimetics may be of therapeutic use.

Shock. 2002 Sep;18(3):230-5. Wolters Kluwer Lippincott Williams & Wilkins Links

Superoxide dismutase mimetics with catalase activity reduce the organ injury in hemorrhagic shock.

- Izumi M, McDonald MC, Sharpe MA, Chatterjee PK, Thiemermann C. Department of Experimental Medicine & Nephrology, The William Harvey Research Institute, St. Bartholomew's and the Royal London School of Medicine & Dentistry, Queen Mary, University of London, United Kingdom.

Reactive oxygen species (ROS) contribute to the multiple organ failure (MOF) in hemorrhagic shock. Here we investigate the effects of two superoxide dismutase (SOD) mimetics with catalase activity (EUK-8 and EUK-134) on the circulatory failure and the organ injury and dysfunction associated with hemorrhagic shock in the anesthetised rat. Hemorrhage (sufficient to lower mean arterial blood pressure to 45 mmHg for 90 min) and subsequent resuscitation with shed blood resulted (within 4 h after resuscitation) in a delayed fall in blood pressure, liver injury and renal dysfunction as well as pancreatic injury. Treatment of rats on resuscitation with EUK-8 (3 mg/kg i.v. bolus followed by 3 mg/kg/h i.v. infusion) significantly attenuated liver injury, renal dysfunction and pancreatic injury caused by hemorrhage and resuscitation. Administration of EUK-134 (3 mg/kg i.v. bolus followed by 3 mg/kg/h) reduced the liver injury and renal dysfunction (but not the pancreatic injury) caused by hemorrhagic shock. However, neither EUK-8 nor EUK-134 reduced the delayed circulatory failure associated with hemorrhagic shock. *Thus, we propose that an enhanced formation of ROS contributes to the MOF in hemorrhagic shock, and that membrane-permeable SOD-mimetics with catalase activity, such as EUK-8 or EUK-134, may represent a novel therapeutic approach for the therapy of hemorrhagic shock.*

J Pharmacol Exp Ther. 2004 Jun;309(3):869-78. Epub 2004 Feb 26. Full Text FREE J Pharma Exp Ther Links

A newly identified role for superoxide in inflammatory pain.

- Wang ZQ, Porreca F, Cuzzocrea S, Galen K, Lightfoot R, Masini E, Muscoli ,
- Mollace V, Ndengele M, Ischiropoulos H, Salvemini D. Department of Biological and Pharmacological Research, Metaphore Pharmaceuticals, 1910 Innerbelt Business Center Drive, St. Louis, MO 63114, USA.

Novel classes of pain-relieving molecules are needed to fill the void between nonsteroidal anti-inflammatory agents and narcotics. Our studies have identified superoxide as a novel mediator of hyperalgesia (clinically defined as an augmented sensitivity to painful stimuli) and have exposed potential pathways through which this radical modulates the hyperalgesic response. The role of superoxide in pain was elucidated using a superoxide dismutase mimetic, M40403 [a manganese(II) complex with a bis(cyclo-hexylpyridine-substituted) macrocyclic ligand]. Intraplantar injection of carrageenan in rats led to time-dependent development of peripheral

inflammation [measured parameters of inflammation included paw edema, cytokine release in the paw exudates, nitrotyrosine formation (a marker of peroxynitrite formation and oxidative stress), and poly-ADP-ribose-polymerase activation (the nuclear enzyme activated by superoxide/peroxynitrite)] and hyperalgesia. M40403 blocked all measured parameters of inflammation and hyperalgesia. Furthermore, when given therapeutically (2 h after the induction of hyperalgesia) either by intravenous or intrathecal administration, M40403 but not its inactive congener M40404 inhibited hyperalgesia with a rapid onset of action. Our results also show that, at the level of the spinal cord and time of peak hyperalgesia, endogenous manganese superoxide dismutase was nitrated and subsequently deactivated, losing its capacity to remove superoxide. The antihyperalgesic effects of M40403 were not reversed by naloxone excluding the potential involvement of an opiate pathway. *Collectively, these studies have unraveled a critical role for superoxide in the nociceptive signaling cascade both peripherally and centrally. The discovery of this pathway opens a new therapeutic strategy for the development of novel nonnarcotic antihyperalgesic agents.*

Arthritis Rheum. 2001 Dec;44(12):2909-21. Links

Amelioration of joint disease in a rat model of collagen-induced arthritis by M40403, a superoxide dismutase mimetic.

- Salvemini D, Mazzon E, Dugo L, Serraino I, De Sarro A, Caputi AP, Cuzzocrea S.
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OBJECTIVE: To investigate the effects of M40403, a synthetic mimetic of superoxide dismutase (SOD), on collagen-induced arthritis (CIA) in rats. **METHODS**: CIA was elicited in Lewis rats by intradermal injection of 100 microl of an emulsion of bovine type II collagen (CII) in Freund's incomplete adjuvant at the base of the tail. A second injection was given on day 21. **RESULTS**: Immunization induced an erosive arthritis of the hind paws. Macroscopic evidence of CIA first appeared as periarticular erythema and edema in the hind paws by days 24-26 after the first injection, with a 100% incidence by days 27. Severity progressed over a 35-day period. Radiography revealed soft tissue swelling and focal resorption of bone, together with osteophyte formation in the tibiotarsal joint. Histopathologic features included erosion of the articular cartilage at the joint margins and subchondral bone resorption associated with bone-derived multinucleated cell-containing granulomatous lesions. Treatment with M40403 (2-10 mg/kg/day) starting at the onset of arthritis (day 25) ameliorated the clinical signs on days 26-35 and improved the histologic findings in the joint and paw. Immunohistochemical analysis for nitrotyrosine (a marker of peroxynitrite formation) and poly(ADP-ribose) polymerase (PARP; a nuclear enzyme activated by DNA single-strand damage) revealed positive staining in the inflamed joints of CII-treated rats, suggestive of the formation of peroxynitrite and DNA damage, both of which were markedly reduced by M40403 treatment. Radiographic evidence of protection from bone resorption, osteophyte formation, and soft tissue swelling was apparent in the tibiotarsal joints of M40403-treated rats. Arthritic rats treated with M40403 gained weight at the same rate and to the same extent as normal, nonarthritic rats. **CONCLUSION:** This study shows that a low molecular weight mimetic of SOD, M40403, attenuates the degree of chronic inflammation, tissue damage, and bone damage associated with CIA in the rat*, and supports the possible use of SOD mimetics as therapeutic agents for the management of chronic diseases such as rheumatoid arthritis.*

Br J Pharmacol. 1998 Jun;124(4):719-28. Links

Loss of endothelium-derived nitric oxide in rabbit aorta by oxidant stress: restoration by superoxide dismutase mimetics.

- **MacKenzie A**, **Martin W**. Clinical Research Initiative, Institute of Biomedical & Life Sciences, University of Glasgow.

Structurally distinct superoxide dismutase (SOD) mimetics were examined for their ability to protect nitric oxide (NO) from destruction by oxidant stress in rabbit aorta. 2. These were the spin traps, PTIYO (4-phenyl-2,2,5,5-tetramethyl imidazolin-1-yloxy-5-oxide), tempol (4-hydroxy 2,2,6,6,-tetramethylpiperidine-1-oxyl) and tiron (4,5-dihydroxy-1,3-benzene-disulphonic acid), the metal salts, CuSO4 and MnCl2, and the metal-based agents CuDIPS (Cu (II)-[diisopropylsalicylate]2) and MnTMPyP (Mn (III) tetrakis [1-methyl-4-pyridyl]porphyrin). 3. Oxidant stress was generated in isolated aortic rings by inactivating endogenous Cu/Zn SOD with diethyldithiocarbamate (DETCA; 60 min) either alone at 3 mM or at 0.3 mM in combination with superoxide generation using xanthine oxidase (XO; 4.8 mu ml(-1)) and hypoxanthine (HX; 0.1 mM). 4. Acetylcholine (ACh)-induced relaxation was inhibited by DETCA (3 mM, 60 min) and was not restored by exogenous SOD (250 u ml(-1)), suggesting the oxidant stress was intracellular. MnTMPyP (600 microM and 1 mM) and MnCl2 (100 microM) were the only agents to reverse the blockade of ACh-induced relaxation. 5. Addition of XO/HX to DETCA (0.3 mM)-treated tissues powerfully impaired ACh-induced relaxation and exogenous SOD (250 u ml(-1)) fully reversed the blockade, suggesting the oxidant stress was extracellular. CuDIPS (0.1-3 microM), CuSO4 (0.3-3 microM), MnCl2 (1-100 microM) and MnTMPyP (100-600 microM) also reversed blockade powerfully, tempol (30 microM-1 mM) and tiron (0.3-10 mM) reversed blockade weakly and PTIYO (10-300 microM) enhanced the blockade. 6. Thus, MnTMPyP was the only SOD mimetic to restore NO-dependent relaxation in conditions of both extracellular and intracellular oxidant stress. *This agent may, therefore, provide a lead in the development of SOD mimetics for the treatment of pathologies associated with oxidant stress.*

Free Radic Biol Med. 1992;13(2):169-81. Links

Oxygen radicals in ulcerative colitis.

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This article reviews the pathophysiologic concept that superoxide and hydrogen peroxide, generated by activated leukocytes, together with low-molecular-weight chelate iron derived from fecal sources and from denatured hemoglobin, amplify the inflammatory response and subsequent mucosal damage in patients with active episodes of ulcerative colitis. The putative pathogenic mechanisms reviewed are as follows: (1) Dietary iron is concentrated in fecal material owing to normally limited iron absorption. (2) Mucosal bleeding, characteristic of ulcerative colitis, as well as supplemental oral iron therapy for chronic anemia, further conspire to maintain or elevate mucosal iron concentration in colitis. (3) Fenton chemistry, driven especially by leukocyte-generated superoxide and hydrogen peroxide, leads to formation of hydroxyl radicals. (4) The resultant oxidative stress leads to the extension and propagation of crypt abscesses, either through direct membrane disruption by lipid peroxidation or through generation of secondary toxic oxidants such as chloramines.

(5) Chemotactic products of lipid peroxidation, including 4-hydroxynonenal, provide positive feedback to accelerate this inflammatory/oxidative process, leading to acute exacerbations of the disease. (6) Other oxidized products, such as oxidized tryptophan metabolites, created by free radical mechanisms in or near the mucosa, may act as carcinogens or tumor promotors that contribute to the exceedingly high incidence of colon carcinoma in patients suffering from chronic ulcerative colitis. *In this way, self-sustaining cycles of oxidant formation may amplify flare-ups of inflammation and mucosal injury in ulcerative colitis. This concept, if proved correct by subsequent research, would provide a rationale for several novel clinical approaches to the management of ulcerative colitis, including use of SOD mimetics, iron chelators, and chain-breaking antioxidants.*

11. Antioxidant enzymes and disordered vision

Pathophysiology. 2006 Aug;13(3):151-62. Epub 2006 Jun 12. ELSEVIER FULL-TEXT ARTICLE Links

Oxidative stress in cataracts.

- **Vinson JA**. Department of Chemistry, University of Scranton, Linden and Monroe Streets, Scranton, PA 18510, USA.

Oxidative stress is the result of an imbalance of antioxidants and pro-oxidants. Since toxic free radicals are the result of normal metabolism, their destruction is imperative. *Cataracts are the leading cause of blindness worldwide. Opacity of the lens is a direct result of oxidative stress*. Cataracts occur primarily due to age, but also are *common in diabetes where superoxide in the mitochondria is elevated as a result of hyperglycemia.* This review will investigate the risk factors of cataract including diet (vitamins, fat and alcohol) as well as UV light and diabetes. The pathophysiology of lens opacification will be discussed and related to the biochemistry, especially during the aging process and in diabetes. Animal and human supplemental antioxidant studies will be reviewed and the mechanisms discussed for cataract prevention and treatment. New genetic engineering approaches to overexpress antioxidant enzymes have given intriguing results and show promise. Lastly, a new approach to target mitochondrial superoxide with antioxidant molecules will be outlined.

Ophthalmic Res. 1982;14(3):167-75. Links

Photoperoxidation in lens and cataract formation: preventive role of superoxide dismutase, catalase and vitamin C.

- **Varma SD**, **Srivastava VK**, **Richards RD**.

Exposure of rat lens to fluorescent daylight (150 ft candles) under tissue culture conditions led to a substantial lipid peroxidation as evidenced by the formation of malondialdehyde (MDA). MDA content of lenses incubated overnight in presence of such light was approximately sixfold of that in the control lenses cultured in the dark. These cultures were maintained in physiological medium resembling aqueous humor which does not contain any additional photoactive component. Thus, the lens in its physiological surroundings is susceptible to photoperoxidation by light of wavelengths which freely penetrate the eye. *Photoperoxidation*

could be thwarted by superoxide dismutase, catalase, and ascorbate, suggesting that the observed peroxidative degradation is initiated by photocatalytic generation of superoxide and its subsequent derivation to other potent oxidants. These studies provide for the first time suggestive evidence that senile cataract development may in part be *linked to the in vivo photochemical generation of superoxide and other potent oxidants in the aqueous humor and lens derived from the ambient oxygen and light*; and ascorbate which is maintained at high levels in this fluid by virtue of its active transport from plasma, is physiologically important in preventing the deleterious action of these potent oxidants. The studies thus indicate for the first time the possibilities of a hitherto unrecognized role of ascorbate against cataracts and other age-, light- and oxygen-dependent ocular abnormalities*. In addition, the study re-emphasizes the role of tissue catalase and superoxide dismutase in the prevention of photoperoxidative damages to the tissue.*

Doc Ophthalmol. 2003 Mar;106(2):129-36. SpringerLink FULL-TEXT ARTICLE Links

Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration.

- **Evereklioglu C**, **Er H**, **Doganay S**, **Cekmen M**, **Turkoz Y**, **Otlu B**, **Ozerol E**. Department of Ophthalmology, Erciyes University Medical Faculty, Kayseri, Turkey. evereklioglu@hotmail.com

BACKGROUND: Nitric oxide (NO), hydroxyl radical (OH*), superoxide anion (O2-) and hydrogen peroxide (H2O2) are free-radicals released in oxidative stress. Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) are antioxidant enzymes, mediating defense against oxidative stress. *Excess NO and/or defective antioxidants cause lipid peroxidation, cellular dysfunction and death. Age-related maculopathy (ARM) or degeneration (ARMD) is the leading cause of irreversible blindness in developed countries*. The etiology is unclear and the molecular factors contributing this disease remain to be specified. **AIMS:** This multicenter, double-blind, cross-sectional study aimed to investigate plasma NO and lipid peroxidation levels with relation to antioxidant enzyme activities in erythrocyte and plasma of patients with ARMD compared with healthy control subjects. **METHODS**: NO, lipid peroxidation (measured as plasma malondialdehyde [MDA] levels) and the catalytic activity of SOD, GSHPx and CAT were measured in a group of 41 patients with maculopathy (19 men, 22 women; 67.12 +/- 3.70 years) and compared with 25 age- and sex-matched healthy control subjects without maculopathy (12 men, 13 women; 68.04 +/- 3.02 years). NO and MDA levels were measured in plasma, CAT in red blood cells (RBCs), and SOD and GSHPx in both plasma and RBCs. Color fundus photographs were used to assess the presence of maculopathy, and the patients were divided into two groups using clinical examination and grading of photographs; early-ARM (n = 22) and late-ARMD (n = 19). **RESULTS:** All patients with maculopathy had significantly ($p < 0.001$) higher plasma NO levels over control subjects (mean +/- SD, 48.58 +/- 8.81 vs. 28.22 +/- 3.39 micromol/l). Plasma MDA levels in patients and control subjects were 4.99 +/- 1.00 and 2.16 +/- 0.24 micromol/l, respectively, and the difference was significant ($p < 0.001$). On the other hand, SOD and GSHPx activities were significantly lower in both RBCs and plasma of patients with maculopathy than in control subjects (RBCs-SOD, 3509.30 +/- 478.22 vs. 5033.30 +/- 363.98 U/g Hb, $p < 0.001$; plasma-SOD, 560.95 +/- 52.52 vs. 704.76 +/- 24.59 U/g protein, $p < 0.001$; RBCs-GSHPx, 663.43 +/- 41.74 vs. 748.80 +/- 25.50 U/g Hb, $p < 0.001$; plasma-GSHPx, 98.26 +/- 15.67 vs. 131.80 +/- 8.73 U/g protein, $p < 0.001$). RBCs-CAT levels were not different between groups (131.68 +/- 12.89 vs. 133.00 +/- 13.29 k/g Hb, $p = 0.811$). Late-ARMD patients had significantly lower antioxidant enzyme levels and higher MDA levels

when compared with early-ARM patients (for each, $p < 0.001$). In addition, plasma NO and MDA levels were negatively correlated with SOD and GSHPx activities. **CONCLUSIONS**: *This study demonstrated for the first time that NO, the most abundant free-radical in the body, might be implicated in the pathophysiology of ARMD in association with decreased antioxidant enzymes and increased lipid peroxidation status.*

12. Mitochondrial dysfunction and Ageing

Oxidative stress in human aging and mitochondrial disease-consequences of defective mitochondrial respiration and impaired antioxidant enzyme system.

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  Respiratory function of mitochondria is compromised in aging human tissues and severely impaired in the patients with mitochondrial disease. A wide spectrum of mitochondrial DNA (mtDNA) mutations has been established to associate with mitochondrial diseases. Some of these mtDNA mutations also occur in various human tissues in an age-dependent manner. These mtDNA mutations cause defects in the respiratory chain due to impairment of the gene expression and structure of respiratory chain polypeptides that are encoded by the mitochondrial genome. *Since defective mitochondria generate more reactive oxygen species (ROS) such as O2- and H2O2 via electron leak, we hypothesized that oxidative stress is a contributory factor for aging and mitochondrial disease*. This hypothesis has been supported by the findings that oxidative stress and oxidative damage in tissues and culture cells are increased in elderly subjects and patients with mitochondrial diseases. Another line of supporting evidence is our recent finding that the *enzyme activities of Cu,Zn-SOD, catalase and glutathione peroxidase (GPx) decrease with age in skin fibroblasts. By contrast, Mn-SOD activity increases up to 65 years of age and then slightly declines thereafter.* On the other hand, we observed that the RNA, protein and activity levels of Mn-SOD are increased two- to three-fold in skin fibroblasts of the patients with CPEO syndrome but are dramatically decreased in patients with MELAS or MERRF syndrome. However, the other antioxidant enzymes did not change in the same manner. The imbalance in the expression of these antioxidant enzymes indicates that the production of ROS is in excess of their removal, which in turn may elicit an elevation of oxidative stress in the fibroblasts. *Indeed, it was found that intracellular levels of H2O2 and oxidative damage to DNA and lipids in skin fibroblasts from elderly subjects or patients with mitochondrial diseases are significantly increased as compared to those of age-matched controls*. Furthermore, Mn-SOD or GPx-1 gene knockout mice were found to display neurological disorders and enhanced oxidative damage similar to those observed in the patients with mitochondrial disease. These observations are reviewed in this article to support that oxidative stress elicited by defective respiratory function and impaired antioxidant enzyme system plays a key role in the pathophysiology of mitochondrial disease and human aging.

Ann N Y Acad Sci. 1998 Nov 20;854:155-70. Full Text Ann N Y Acad Sci Links

Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function.

- **Wei YH**, **Lu CY**, **Lee HC**, **Pang CY**, **Ma YS.** Department of Biochemistry, School of Life Science, National Yang-Ming University, Taipei, Taiwan, Republic of China. joeman@mailsrv.ym.edu.tw

Mitochondrial respiration and oxidative phosphorylation are gradually uncoupled, and the activities of the respiratory enzymes are concomitantly decreased in various human tissues upon aging. An immediate consequence of such gradual impairment of the respiratory function is the increase in the production of the reactive oxygen species (ROS) and free radicals in the mitochondria through the increased electron leak of the electron transport chain. Moreover, the intracellular levels of antioxidants and free radical scavenging enzymes are gradually altered. *These two compounding factors lead to an age-dependent increase in the fraction of the ROS and free radical that may escape the defense mechanism and cause oxidative damage to various biomolecules in tissue cells. A growing body of evidence has established that the levels of ROS and oxidative damage to lipids, proteins, and nucleic acids are significantly increased with age in animal and human tissues*. The mitochondrial DNA (mtDNA), although not protected by histones or DNA-binding proteins, is susceptible to oxidative damage by the ever-increasing levels of ROS and free radicals in the mitochondrial matrix. In the past few years, oxidative modification (formation of 8-hydroxy-2'-deoxyguanosine) and large-scale deletion and point mutation of mtDNA have been found to increase exponentially with age in various human tissues. *The respiratory enzymes containing the mutant mtDNA-encoded defective protein subunits inevitably exhibit impaired respiratory function and thereby increase electron leak and ROS production, which in turn elevates the oxidative stress and oxidative damage of the mitochondria. This vicious cycle operates in different tissue cells at different rates and thereby leads to the differential accumulation of mutation and oxidative damage to mtDNA in human aging.* This may also play some role in the pathogenesis of degenerative diseases and the age-dependent progression of the clinical course of mitochondrial diseases.

13. Mutagenesis / Cancer and SOD

Free Radic Biol Med. 2006 Jul 15;41(2):226-37. Epub 2006 May 4. ELSEVIER FULL-TEXT ARTICLE Links

Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth.

- **Weydert CJ**, **Waugh TA**, **Ritchie JM**, **Iyer KS**, **Smith JL**, **Li L**, **Spitz DR**, **Oberley LW.** Free Radical and Radiation Biology Program, Department of Radiation Oncology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA 52242, USA. christine.weydert@uiowa.edu

We have studied the effects of overexpression of superoxide dismutase (SOD), a tumor suppressor protein that dismutes superoxide radical to H2O2, on breast cancer cell growth in

vitro and xenograft growth in vivo. No previous work has directly compared the growth-suppressive effects of manganese SOD (MnSOD) and copper-zinc SOD (CuZnSOD). We hypothesized that either adenoviral MnSOD (AdMnSOD) or adenoviral CuZnSOD (AdCuZnSOD) gene therapy would suppress the growth of human breast cancer cells. After determining the antioxidant profiles of three human breast cell lines, MCF 10A, MDA-MB231, and MCF-7, we measured the effects of MnSOD or CuZnSOD overexpression on cell growth and survival in vitro and in vivo. Results demonstrated that infection with AdMnSOD or AdCuZnSOD increased the activity of the respective enzyme in all three cell lines. In vitro, overexpression of MnSOD or CuZnSOD decreased not only cell growth but also clonogenic survival in a dose- and transgene-dependent manner. In vivo, treatment of tumors with AdMnSOD or AdCuZnSOD decreased xenograft growth compared to controls. *The first direct comparison of MnSOD to CuZnSOD overexpression indicated that CuZnSOD and MnSOD were similarly effective at suppressing cancer cell growth.*

Biomarkers. 2006 Nov-Dec;11(6):574-84. MetaPress Links

Superoxide dismutase in gastric adenocarcinoma: is it a clinical biomarker in the development of cancer?

- **Monari M, Trinchero A, Calabrese C, Cattani O, Serrazanetti GP, Foschi J, Fabbri A, Zahlane D, Di Febo G, Tonini V, Cervellera M, Tosi MR, Tugnoli V.** Dipartimento di Biochimica G. Moruzzi, Universita di Bologna, Bologna, Italy. mmonari@vet.unibo.it

Gastric cancer is the second most common cancer worldwide. The involvement of reactive oxygen species (ROS) in the pathogenesis of gastric malignancies is well known. Many human tumours have shown significant changes in the activity and expression of superoxide dismutase (SOD), which might be correlated with clinical-pathological parameters for the prognosis of human carcinoma. The aim of this study is the detection of MnSOD and CuZnSOD activity and their expression in gastric adenocarcinoma and healthy tissues. Gastric samples (adenocarcinoma and healthy tissues) harvested during endoscopy or resected during surgery were used to determine MnSOD and CuZnSOD activity and expression by spectrophotometric and Western blotting assays. *The total SOD activity was significantly higher ($p<0.05$) in healthy mucosa with respect to gastric adenocarcinomas*. No differences were found in MnSOD activity and, on the contrary, CuZnSOD activity was significantly lower ($p<0.001$) in cancer samples with respect to normal mucosa. The rate of MnSOD/CuZnSOD activity in adenocarcinoma was over ninefold higher than that registered in healthy tissues ($p<0.05$). Moreover, in adenocarcinoma MnSOD activity represented the 83% of total SOD with respect to healthy tissues where the ratio was 52% ($p<0.001$). On the contrary, in cancer tissues, CuZnSOD activity accounted for only 17% of the total SOD ($p<0.001$ if compared with the values recorded in normal mucosa). After immunoblotting, MnSOD was more expressed in adenocarcinoma with respect to normal mucosa ($p<0.001$), while CuZnSOD was similarly expressed in adenocarcinoma and healthy tissues. The SOD activity assay might provide a specific and sensitive method of analysis that allows the differentiation of healthy tissue from tumour tissue. *The MnSOD to CuZnSOD activity ratio, and the ratio between these two isoforms and total SOD, presented in this preliminary study might be considered in the identification of cancerous from healthy control tissue.*

Mol Cancer Ther. 2003 Apr;2(4):361-9. Full Text FREE Mol Cancer Ther Links

Suppression of the malignant phenotype in human pancreatic cancer cells by the overexpression of manganese superoxide dismutase.

- **Weydert C**, **Roling B**, **Liu J**, **Hinkhouse MM**, **Ritchie JM**, **Oberley LW**, **Cullen JJ**. Department of Radiation Oncology, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA.

Cells contain a large number of antioxidants to prevent or repair the damage caused by reactive oxygen species. One component of the antioxidant system, manganese superoxide dismutase *(MnSOD), is localized in the mitochondria, and the levels of this protein have been previously shown to inversely correlate with pancreatic cancer cell growth.* The aim of the present study was to determine whether MnSOD overexpression could suppress the in vitro and in vivo malignant phenotype of a human pancreatic cancer cell line. Tumor cell behavior was determined in the pancreatic cancer cell line MIA PaCa-2 by examining cell growth, plating efficiency, and anchorage-independent growth in soft agar. MnSOD was overexpressed in the pancreatic cancer cell line MIA PaCa-2 by infection with an adenovirus-MnSOD construct. Cells were also injected s.c. in nude mice and tumor volume was calculated. Single and multiple direct injections of the adenoviral MnSOD construct (10(9) plaque-forming units) were delivered to the tumor. Increases in MnSOD immunoreactivity and activity were seen after transduction with the adenovirus-MnSOD construct. Increasing MnSOD levels correlated with increased doubling time. Cell growth, plating efficiency, and growth in soft agar decreased with increasing amounts of the adenovirus MnSOD construct. Tumors grew slower and survival was increased in nude mice injected with the adenoviral MnSOD construct compared with the parental cell line, whereas multiple injections of the adenoviral MnSOD construct further inhibited tumor cell growth and extended survival. *These results suggest that MnSOD may be a tumor suppressor gene in human pancreatic cancer.* Delivery of the MnSOD gene may prove beneficial for suppression of pancreatic cancer growth.