

The Benefits of **melonx**



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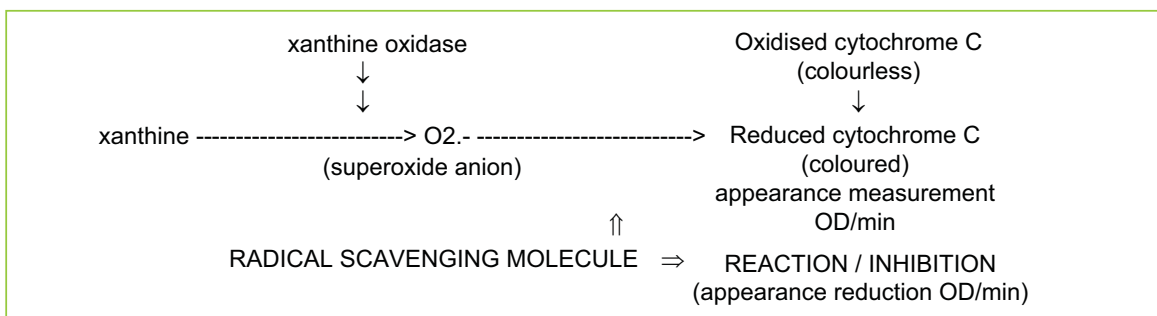
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Protective Effect Against ROS (Reactive Oxygen Species)

Test Principle

Determination of the radical scavenging effect is based on the inhibition or the decrease in the speed of the reduction of an uncoloured oxidised molecule which is coloured when it is reduced, cytochrome C for example, by adding to the reactive medium the radical scavenging molecule to study. The ROS is generated, for example by the action of xanthine oxidase on xanthine (production of superoxide anion). In the absence of a molecule able to capture it, the ROS leads to the reduction of the cytochrome C. The appearance of reduced molecule is monitored using a spectrophotometer (at 550 nm for cytochrome C) in the presence of radical scavenging molecules.

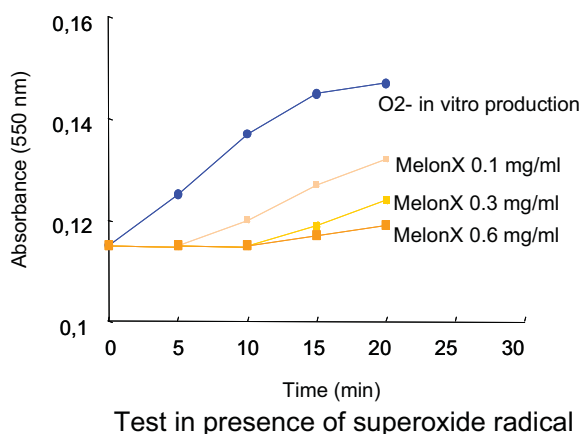


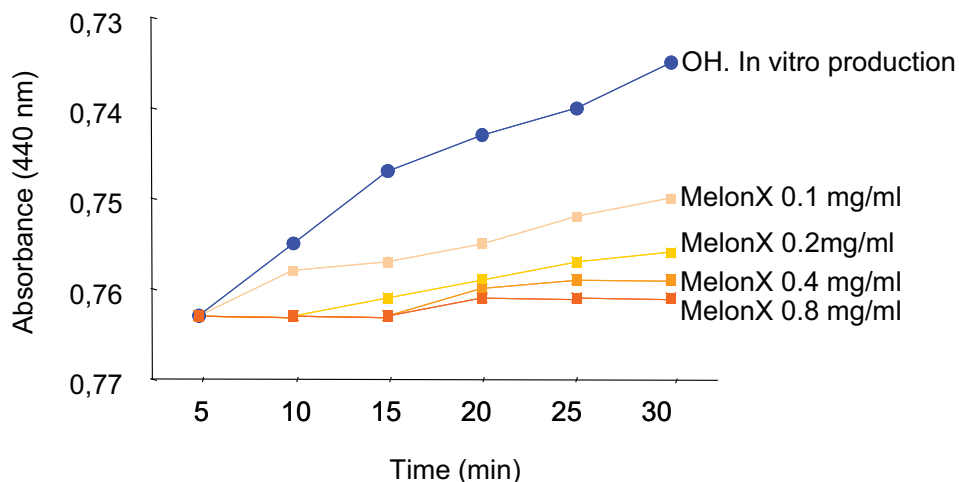
Test Description

3 tests have been done:

- 3 concentrations of MelonX in presence of superoxide radical : 0.1, 0.3 and 0.6 mg/ml respectively corresponding to 7, 21 and 42 UI/mg of SOD activity.
- 4 concentrations of MelonX in presence of hydroxyl radical : 0.1, 0.2, 0.4 and 0.8 mg/ml respectively corresponding to 7, 14, 28 and 56 UI/mg of SOD activity.
- 4 concentrations of MelonX in presence of hydrogen peroxide : 0.1, 0.2, 0.4 and 0.8 mg/ml respectively corresponding to 7, 14, 28 and 56 UI/mg of SOD activity.

Results

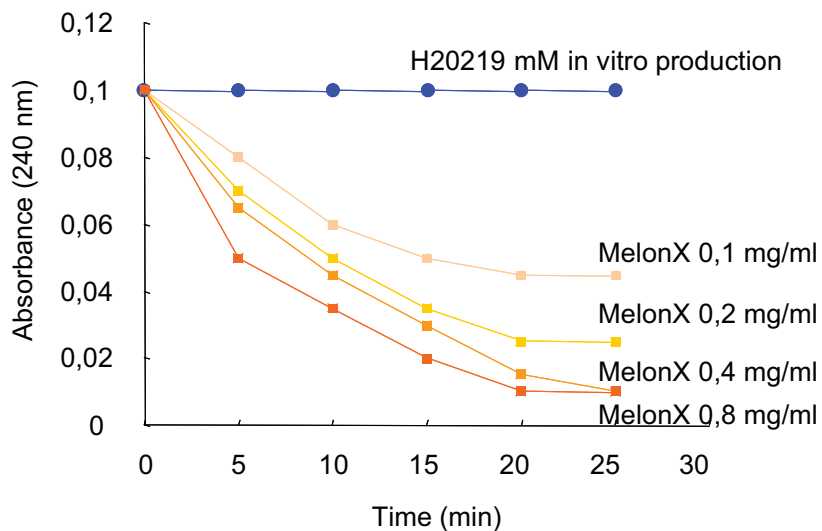




Test in presence of hydroxyl radical

Test in presence of hydrogen peroxide

The results have shown that MelonX has a protective effect against the 3 primary free radicals or reactive oxygen species. MelonX has an effective antioxidant activity in vitro on the 3 reactive oxygen species at a minimum concentration of 1‰, corresponding to 7 UI/ml. MelonX has no prooxidant activity at higher concentrations.



Test in presence of hydrogen peroxide

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Protective Effect Against UV Radiations

Environmental stress factors (UV, pollutants...) attack skin cells daily. This oxidative stress often induces the death of keratinocytes by apoptosis as well as the release of soluble mediators that contribute to the development of a local inflammation. The protective effect of MelonX was evaluated under an oxidative stress : the UV exposure.

In Vitro Protective Effect On Keratinocytes

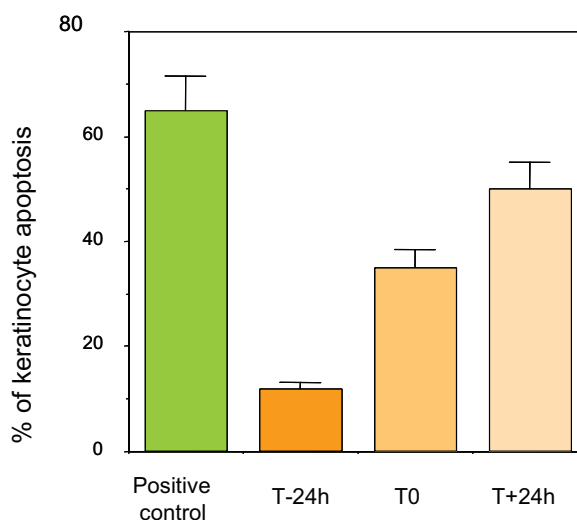
Description

MelonX was introduced at 2 mg/ml, corresponding to 30 UI SOD/ml, in a human keratinocyte culture medium at 3 times: 24 hours before the UV stress (preventive effect : T-24h), in the same time as exposure (extemporaneous : T0) and 24 hours after the UV exposure (curative effect : T+24h).

The keratinocyte apoptosis was evaluated 72h after the UV irradiation by fluorescent microscopy. The release of some pro-inflammatory mediators as cytokines (TNF, IL-6) and NO were followed 48 hours after the UV exposure.

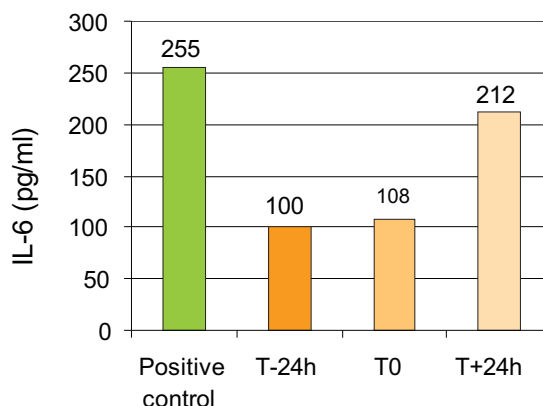
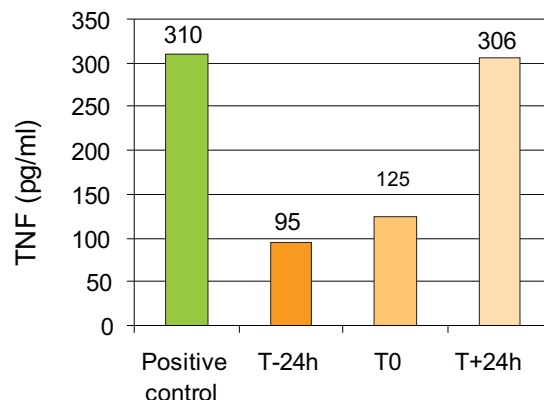
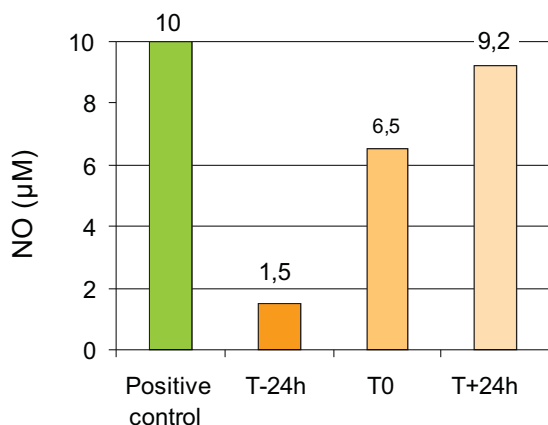
Results On Apoptosis

MelonX clearly reduced UV induced apoptosis of keratinocytes when it is introduced 24 hours before the UV exposure, greatly reduced it when it is introduced extemporaneously and has no significant effect when it is introduced 24 hours after UV irradiation.



Results On Production Of Pro-inflammatory Mediators

A reduction in the production of pro-inflammatory cytokines and NO is observed when MelonX is added as a preventive. However, this effect is lessened when MelonX is added extemporaneously and has no significant effect when it is added after UV irradiation. MelonX has a preventive effect on UV exposure-induced inflammation by limiting the production of pro-inflammatory mediators.



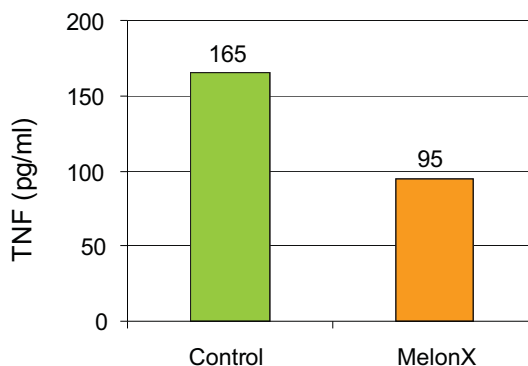
In vivo protective effect on nude mice

Description

The protective effect of MelonX , orally absorbed, against UV-induced skin injury was tested in vivo. Nude mice, with transplants of human skin explants, were fed with 0.7 mg of MelonX per day, corresponding to 10 IU SOD/day, during two weeks before UVB irradiation. Only the human skin was irradiated and taken into account in the evaluation of the product efficacy. The release of TNF-α was followed as an proinflammatory marker.

Results

The oral absorption of MelonX reduces markedly the TNF-α production - around 40%. The oral absorption of MelonX has a preventive effect on skin cells against UV radiation oxidative stress.



Discussion

The production of pro-inflammatory mediators and the apoptotic cell death are linked to an important production of ROS arising from the interaction between the UV rays and the oxygen. When the physiological antioxidant pool is not sufficient to prevent the oxidative damage caused by UV rays, cellular deterioration accompanied by an inflammatory reaction occurs. MelonX added before UV irradiation, inhibits the development of proinflammatory mediators and simultaneously reduces the entry of apoptotic cells. In this study, it is clear that MelonX has a protective effect on cellular metabolism.

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Protective Effect Against Stress

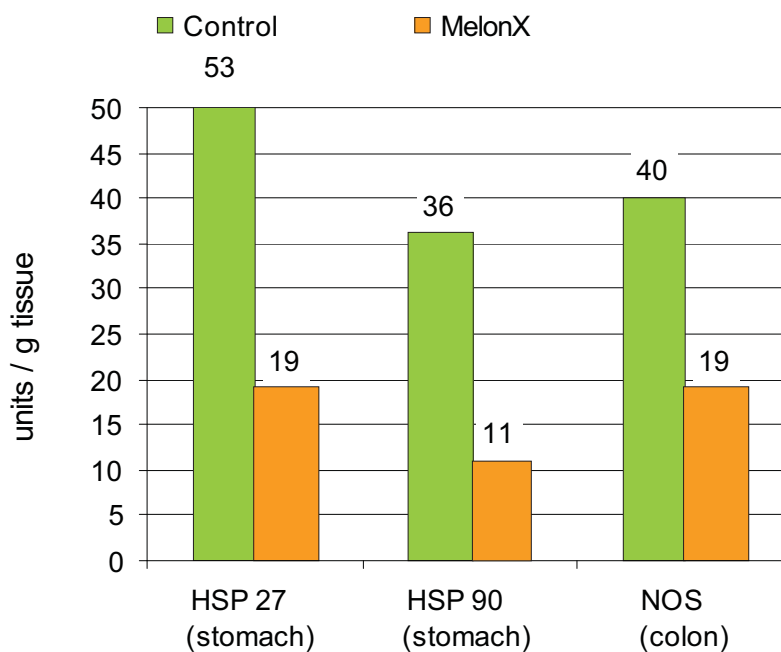
Physical, physiological or psychological stress is an frequent state in our actual way of life. At the cellular level, these stress is responsible for inflammatory reactions caused by an increase of reactive oxygen species. SOD is highly implicated in the fight against ROS and then in the prevention of inflammatory reactions. In order to show the protective effect of MelonX against stress, in partnership with INRA UMRVP (Saint-Gilles, France), we carry out an in vivo test on piglets in stressful conditions : the weaning period.

Description

Piglet feed was supplemented with 0.08 mg/kg BW/day of MelonX 2 days after weaning, corresponding to 1.2 UI SOD/kg BW/day. HSP (heat shock proteins) and NOS (NO synthase) were evaluated in gastrointestinal tissues as markers of oxidative stress, 14 days after weaning.

Results

MelonX markedly reduce –around 50%– the tissular concentration of HSP and NOS. These molecules are produced by the cell in response to stressful conditions. MelonX have a protective effect on stress deleterious effects.



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Protective Effect Against Allergen

Description

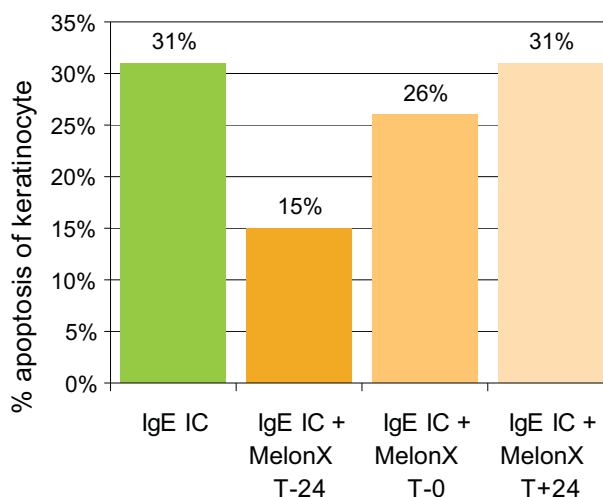
The protective effect of MelonX against allergen substance induced inflammation was carried out on normal human keratinocytes. MelonX was introduced at 10 µg/ml, corresponding to 0.14 UI SOD/ml into keratinocyte culture medium at 3 different times : 24 hours before the allergic stress (preventive effect : T-24h), in the same time as allergic stress (extemporaneous : T0) and 24 hours after the allergic stress (curative effect : T+24h).

In order to simulate an allergic stress-induced inflammatory reaction, keratinocytes were activated using IL-4 (10 ng/ml) for 48 hours to induce the CD23 (IgE low-affinity binding site) and stimulated using IgE Immune Complexes (IgE/anti-IgE). Then, the cells were held in the culture medium for 48 hours.

The apoptosis of keratinocytes and the release of some inflammatory markers (TNFα, IL-6 et NO) were followed.

Results on apoptosis

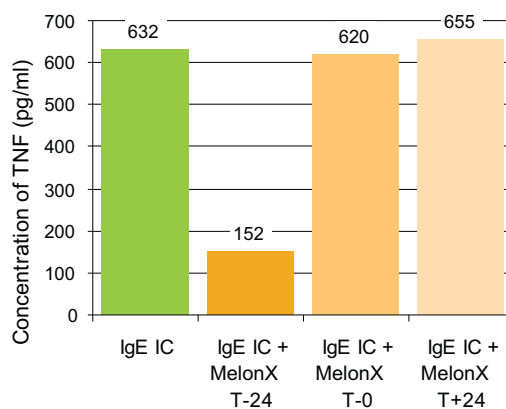
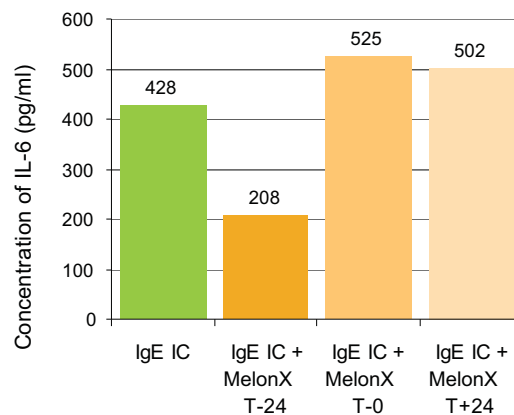
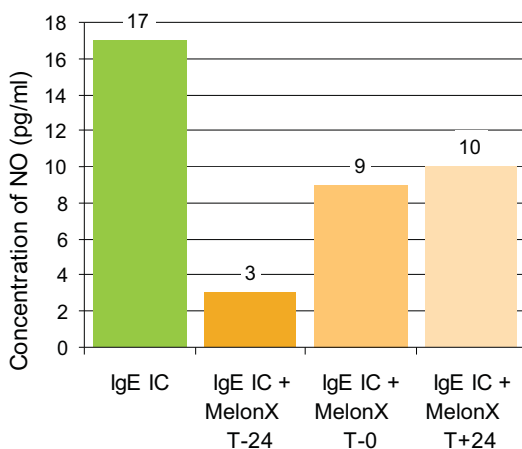
This experiment shows a very clear reduction of cell apoptosis when MelonX is introduced in the culture medium 24h before the allergen stimulation. MelonX has a preventive effect on keratinocytes by limiting the allergic stress-induced apoptosis.



Results on production of pro-inflammatory mediators

A decrease in the production of pro-inflammatory cytokines is observed when MelonX is added to the culture medium 24h before the stimulation whereas the addition at the same time as the stimulation or 24h after the stimulation have no or a very weak effect.

MelonX prevents the allergic stress-induced inflammation by limiting the production of pro-inflammatory mediators.



Discussion

This study shows quite clearly that MelonX exerts a protective effect against the inflammatory reaction induced as an allergic response to an antigenic exposure.

MelonX reduces significantly the cellular apoptosis level induced by an allergic stress.

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SOD & Inflammation

Heat stress

Heat stress (UV for example) are the most frequent. UVB generate free radicals, induce keratinocytes apoptosis and inflammatory reactions characterised by the release of cytokines and the formation of oedemas.

Introduction

Immune system cells play a role in the inflammatory reaction that occurs in the burnt tissues. Physiological changes in the zones damaged by heat are controlled by the local release of inflammatory mediators initiating cascades of reactions which maintain and increase the inflammatory process. Recently results have shown that reactive oxygen species are implicated in these inflammatory reactions, in particular the superoxide radical.

Scavenging superoxide radical, SOD could have an effect on inflammatory reaction induced by heat.

Published studies

Varauer-Uhl et al. (2001) have studied on albinos rabbits the effect of SOD by topical application on burning and oedemas induced by heat or UV. 24h after the heat stress, the size of lesions and oedemas have significantly decrease (25%) in the treated group.

An in vitro study on human keratinocytes have shown the protective role of SOD against deleterious effect caused by UVB (Sasaki et al., 2000). Induced-apoptosis of keratinocytes is decreased by the introduction of antioxidants (SOD, catalase) in the culture medium (Takahashi et al., 2000).

Nevertheless, mechanism of action and transport of SOD into the cells has not yet been described. Only some results have shown that SOD is absorbed by endothelial cells (Muzykantov, 2001) or liver cells by endocytosis (Dini et al., 1996). A study have also shown that after oral administration of SOD, SOD activity is increased in erythrocytes (Regnault et al., 1996).

Allergic stress

Atmospheric pollution, cigarette smoke or allergens (pollen,dust, ...) are unfavourable environmental conditions able to provoke skin or respiratory allergenic reactions.

Introduction

Through immune system, mastocyte cells have an important role in immediate hypersensitivity reaction of allergic reaction. Mastocytes are present in all tissues in direct contact with the environment as skin, lungs or intestine. Mastocytes hold on their surface receptors (FcERI) which have a high affinity with immunoglobulin E (IgE). The fundamental characteristic of allergic reaction is the interaction between IgE present on mastocytes and the allergen that leads to changes in the structure of FcERI receptor, several biochemical events resulting in explosive degranulation of the cell and activation of membrane phospholipases activation resulting in pro-inflammatory mediators liberation.

Reactive oxygen species (ROS) play an important role in this phenomena. In fact, immune response of the cell to allergen substances induces an increase of free radicals and leads mastocytes to release histamine, the main chemical mediator of allergic inflammation (Matés et al., 2000).

Published studies

Nadeem et al. (2003) have shown an increase of free radicals in plasma and a decrease of antioxidant defences (SOD, catalase) in people with asthma. They indicate that an increase of antioxidant defences will be beneficial for them.

Nishida et al. (2002) have shown that SOD is highly implicated in asthmatic reaction because it neutralise free radicals form bronchioalveolar liquid, and then inhibit the development of asthmatic attack.

The effect of SOD on allergic reaction was clearly demonstrated by Assa'ad et al. (1998) on rabbits. Rabbits treated with SOD in a prevention way do not develop asthmatic reaction in response to allergen contrary to control. In conclusion, these works show the positive effect of SOD in the case of allergy.

Joint stress

Introduction

Rheumatoid arthritis, an autoimmune disease that affects the joints, is a major health problem in developed countries such as Europe, Japan and Northern America. Rheumatoid arthritis is characterized by an infiltration of the affected joint by blood-derived cells. In response to activation, these cells generate reactive oxygen species, resulting in an enhanced production of superoxide and peroxynitrite radicals by bloodstream neutrophils and of superoxide radical by monocytes leading to an oxidative stress situation. It seems that NADPH oxidase together with NO synthase are the major sources of superoxide radical and NO in the rheumatoid arthritis neutrophils while in monocytes, superoxide radical is produced by NADPH oxidase and mitochondria.

Reducing the arthritis related inflammation may offer an alternative to current treatments and open a preventive way.

Published studies

Corvo et al. (1999 ; 2000) showed that the delivery of SOD by intravenous or subcutaneous administration in a rat model of arthritis directly at the arthritic sites leads to a reduction of inflammation.

In an other experiment, Sakurai et al (1997) attached covalently the SOD to polyethylene glycol to improve the SOD half-life in the circulation of rodents. They demonstrated that when the SOD half-life is improved, SOD is effective to suppress arthritis in rats. In an in vitro model of blood neutrophils and monocytes of patients with rheumatoid arthritis, Ostrakhovitch and Afanas'ev (2001) showed that in the presence of SOD these neutrophils and monocytes do not produce the superoxide radical. Finally, Zang et al. (2002) used human recombinant SOD in a rat model and strengthened the efficiency of SOD in the treatment of arthritis.

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