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PATENTS ACT 1990

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# PATENT REQUEST : STANDARD PATENT

I/We being the person(s) identified below as the Applicant(s), request the grant of a patent to the person(s) identified below as the Nominated Person(s), for an invention described in the accompanying standard complete specification.

Full application details follow:

[71/70] Applicant(s)/Nominated Person(s):

**Boehringer Ingelheim International GmbH**

of

D-55216 Ingelheim, Germany

[54] Invention Title:

**The use of low doses of manganese superoxide dismutase (Mn-SOD) in the treatment of diseases**

[72] Name(s) of actual inventor(s):

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Basic Convention Application(s) Details:

| [31] Application Number | [33] Country | Code | [32] Date of Application |
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| P 4229801.6 | Germany | DE | 9 September 1992 |

DATED this EIGHTH day of SEPTEMBER 1993

.....
a member of the firm of
DAVIES COLLISON CAVE for
and on behalf of the
applicant(s)

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NOTICE OF ENTITLEMENT

We, **Boehringer Ingelheim International GmbH**, the applicant/Nominated Person in respect of Application No. 46237/93 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person would, on the grant of a patent for the invention to the inventors, be entitled to have the patent assigned to the Nominated Person.

The Nominated Person is entitled to claim priority from the basic application listed on the patent request because the Nominated Person made the basic application, and because that application was the first application made in a Convention country in respect of the invention.

DATED this EIGHTEENTH day of OCTOBER 1993

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a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

(DCC ref: 1615719)

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(54) Title
THE USE OF LOW DOSES OF MANGANESE SUPEROXIDE DISMUTASE (MN-SOD) IN THE TREATMENT OF DISEASES

International Patent Classification(s)
(51)[5] A61K 037/50

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(56) Prior Art Documents
EP 284105

(57) Claim

1. A method of treatment of a human or animal patient suffering from or susceptible to a disease or condition which is caused, partly caused or triggered by oxygen free radicals which comprises administering to the patient 1 to 200 µg of Mn-SOD per kg of bodyweight per day.

14. A pharmaceutical composition in unit dosage form comprising as active ingredient Mn-SOD in association with one or more pharmaceutically acceptable carriers or excipients, wherein each unit dose contains less than 14 mg of Mn-SOD when used to treat a human or animal patient suffering from or susceptible to a disease or condition which is caused, partly caused or triggered by oxygen free radicals.

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COMPLETE SPECIFICATION

NAME OF APPLICANT(S):

**Boehringer Ingelheim International GmbH**

ADDRESS FOR SERVICE:

**DAVIES COLLISON CAVE**
Patent Attorneys
1 Little Collins Street, Melbourne, 3000.

INVENTION TITLE:

The use of low doses of manganese superoxide dismutase (Mn-SOD) in the treatment of diseases

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

It is known that, as a consequence of various biochemical processes in biological systems (e.g. Redox processes in the respiratory chain, oxidations in the cytoplasm), $O_2$. radicals are continuously formed which are highly cytotoxic and may lead to tissue damage. In pathological situations, e.g. in the course of rheumatic disorders, there is some discussion of degradation of collagen and synovial fluid by such radicals (Pasquier, C. et al., Inflammation 8, 27-32, 1984).

Eukaryotic cells contain primarily two forms of superoxide dismutases (SOD), one of which occurs predominantly in the cytosol (Cu/Zn-SOD) whilst the other occurs primarily in the mitochondria (Mn-SOD). In liver mitochondria it has been found that the Mn-enzyme is located in the matrix enclosing the inner membrane, although Mn-SOD has also been detected in the cytosol of the liver cells (Mc Cord J.M. et al. In: Superoxide and Superoxide Dismutases (A.M. Michelson, J.M. Mc Cord, I. Fridovich, eds.) Academic Press, N.Y., 129-138, 1977).

In prokaryotes, there is an Fe-SOD in addition to an Mn-SOD. Fe-SOD has also been detected in algae and protozoa and in some plant species (Bridges, S.M., Salin, M.L., Plant Physiol. 68, 275-278, 1981).

These highly active enzymes catalyse the disportionation $O_2.^- + O_2.^- + 2H^+ \rightarrow H_2O_2 + O_2$ and by this dismutation of the superoxide radicals prevent concentration thereof and hence any cell damage. Apart from the endoplasmic reticulum of the liver, the mitochondrial membranes are regarded as one of the most important sites of $O_2$ production in animal cells, so it is no wonder that mitochondria have their own special SOD(Mn-SOD).

The structural gene of a prokaryotic Mn-SOD (E.coli) has been cloned and the chromosomal sodA-gene

has been located (Touati, D., J. Bact. 155, 1078-1087, 1983).

The 699 bp long nucleotide sequence of a mitochondrial yeast Mn-SOD has been clarified and the primary structure both of the precursor and of the mature protein have been derived therefrom - with molecular weights of 26123 Da for the precursor and 23059 Da for the mature protein (Marres, C.A.M. et al., Eur. J. Biochem. 147, 153-161, 1985). Thus, the Mn and Cu/Zn-SOD (MW=14893, EP-A-138111) differ significantly in their molecular weight.

The complete amino acid sequence of Mn-SOD from human liver has been published by Barra D, and according to this the hMn-SOD is supposed to consist of 196 amino acids (Barra D, et al. J. Biol. Chem. 259, 12595-12601, 1984). Human Cu/Zn-SOD from erythrocytes, on the other hand, consists of 153 amino acids (Jabusch, J.R., et. al. Biochemistry 19, 2310-2316, 1980) and exhibits no sequence homologies to hMn-SOD (Barra, D. et al, see above).

Generally, the superoxide dismutases are credited with a protective function against certain inflammatory processes. In particular, a deficiency of Mn-SOD is thought to be significant in the development of rheumatoid arthritis (Pasquier, C. et al., see above). It is also assumed that SOD has a protective effect against alcohol-induced liver damage (Del Villano B.C. et al., Science 207, 991-993, 1980). Furthermore, SOD is supposed to protect ischaemic tissues (McCord, J.M. & Roy, R.S., Can. J. Physiol. Pharma 60, 1346-1352, 1982) and to have a cardio protective effect, in that the SOD exerts a protective effect on myocardial cells in hypoxia or in tissue damage caused by reperfusion (Omar, B.A., McCord, J.M., J. Mol. Cell. Cardiol 23, 149-159, 1991).

It is generally known that oxygen free radicals play a central part in all kinds of cell and tissue

damage and are one of the main factors, particularly, in triggering inflammatory processes.

In accordance with this finding, attempts have been made to use SOD as a therapeutic agent particularly in diseases which involve inflammatory processes.

However, it has been found that the quantities of Mn-SOD used, in the milligram range (mg/kg of body weight), had either negative effects, which might result in exacerbation of the inflammatory process, or that the Mn-SOD had no effect whatsoever (Omar, B.A. McCord, J.M., Free Radical Biol. Med. 9, 473-478, 1990; Baret, A. et al., Biochem. Pharmacol. 33, 2755-2760, 1984). Accordingly, it is proposed in EP-A 284 105 to use Mn-SOD in a preferred dosage of between 3 and 50 mg/kg of body weight to treat inflammatory diseases, or as described by Nimrod, A. et al., 1989, in: Medical, Biochemical and Chemical Aspects of Free Radicals, O. Hayaishi, E. Niki, M. Kondo, T. Yoshikawa, eds. Elsevier Science Publishers, B.V. Amterdam, pages 743-746, a dosage range of 5 to 50 mg/kg of body weight being specified. It is known from publication by Gorecki, M. et al., Free Rad. Res. Comms. Vols. 12-13, 401-410, 1991, that Mn-SOD has a radio protective effect at a dose of 100 mg/kg i.v. and is supposed to be antiinflammatory within a dosage range from 8 to 20 mg/kg i.v. or at 50 mg/kg s.c.

Surprisingly, it has been found that the use of Mn-SOD in a low dosage range of from 1 to 200 µg/kg of body weight as a daily dose was highly effective in diseases caused by oxygen free radicals, and that only in this dosage range was a cell or cyto protective activity achieved without any harmful effects. The preferred dosage range is from 1 to 100 µg/kg of body weight, particularly from 1 to 50 µg/kg of body weight, these dosages referring to a daily dose. In inflammatory diseases which affect the joints directly, e.g. arthritic processes and synovial inflammatory processes,

(synovitis), doses of 1 to 20 μg, more particularly 1 to 10 μg and most particularly 1 to 5 μg of Mn-SOD, based on the volume of a knee joint of a rat, have proved very effective. The knee joint of an adult rat (of approximately 200 g) has a volume of 100 μl on average (Miao, F.J.-P. et al., J. Pharm. Exp. Ther. 262, 889-895, 1992; Vadas, P. et al., Am. J. Pathol. 134, 807-811, 1989).

According to one aspect of the invention, there is provided a method of treatment of a human or animal patient suffering from or susceptible to a disease or condition which is caused, partly caused or triggered by oxygen free radicals which comprises administering to the patient 1 to 200 μg of Mn-SOD per kg of bodyweight per day.

In an alternative aspect, there is provided a method of treatment of a human or animal patient suffering from or susceptible to an inflammatory disease in a joint which comprises administering to the patient 1 to 20 μg of Mn-SOD per 100 μl of the volume of the joint.

According to a further aspect, there is provided the use of manganese superoxide dismutase (Mn-SOD) for preparing a pharmaceutical preparation for the therapeutic or prophylactic treatment of diseases or conditions in a human or animal patient which are caused, partly caused or triggered by oxygen free radicals, by administration to the patient of 1 to 200 μg of Mn-SOD per kg of body weight per day.

According to a still further aspect, there is provided the use of manganese superoxide dismutase (Mn-SOD) for preparing a pharmaceutical preparation for treating an inflammatory disease in a joint, in a human or animal patient, by administration to the patient of 1 to 20 μg of (Mn-SOD) per 100 μl of the volume of the joint.

The efficacy of the low dose of Mn-SOD according to

the invention was highly significant in animal trials and there is no doubt that this low dosage can also be used to prepare a pharmaceutical preparation for the therapeutic or prophylactic treatment of diseases, especially inflammation, or conditions caused by oxygen free radicals in humans.

Surprisingly, it has also been found that Mn-SOD in the dosage according to the invention, is very much more effective than Cu/Zn-SOD and that high doses of Mn-SOD (> 200 µg/kg or > 25 µg/100 µl of joint volume) and Zn/Cu-SOD in general (100 µg/kg or 5 to 50 µg/100 µl of joint volume) either resulted in an exacerbation of the disease processes or were ineffective.

As for the exacerbation of cell or tissue damage found here at high Mn-SOD doses of above 200 µg/kg or above 25 to 50 µg/100 µl of joint volume in joint inflammation such as arthritic diseases (e.g. synovitis), it can only be assumed that these harmful effects might be caused by an increased formation of $H_2O_2$ as a result of the disproportion reactions of Mn-SOD, whilst hydroxyl radicals might be formed by interactions with reduced transition metals (e.g. Fe, Mn). Hydroxyl radicals of this kind are highly reactive and might rapidly combine with various molecules at the site where they are produced. It is also possible for oxygen radicals to react with nitric oxide (NO) to form peroxynitrite ($NO_3^-$), which results in the formation of hydroxyl radicals through the reaction ($NO_3^- + H^+ \rightarrow NO_3H$) and, according to the subsequent reaction, ($NO_3H \rightarrow OH. + NO_2$. The prolonged vasorelaxant effects of NO caused by overscavenging of $O_2.^-$ by Mn-SOD could lead to extravasation and oedema, which could be responsible for the inflammatory effects, and which were found in the investigations shown here with high doses of Mn-SOD (> 200 µg/kg or > 25-50 µg/100 µl of joint volume). It is also possible that Mn-SOD participates in the reversible reduction of NO into the nitroxyl anion.

With regard to the diseases and conditions to be treated by the dosage according to the invention, which are caused, partly caused or triggered by oxygen free radicals or in which oxygen free radicals display undesirable properties, these include for example all kinds of inflammatory processes (such as for example synovial inflammation, Crohn's disease, colitis, rheumatic processes), tissue damage caused by medical intervention (e.g. reperfusion with subsequent ischaemia) or as a result of mechanical, physical (e.g. radiation) or chemical effects, organ and tissue transplants or arthritic processes. In particular, the inflammations include such processes which are peracute, acute, subacute, chronic or recurring as well as those known as localised, generalised or metastising inflammations regarding their extent and location. These include all kinds of known exsudative granulomatous and proliferative inflammations.

The Mn-SOD to be used in the low dosage according to the invention, can preferably be prepared by the known methods of DNA recombination, the process according to EP-A 282 899 being preferred.

According to a further aspect of the invention, there is provided a pharmaceutical composition in unit dosage from comprising as active ingredient Mn-SOD in association with one or more pharmaceutically acceptable carriers or excipients, wherein each unit dose contains less than 14 mg of Mn-SOD.

Preferably, each unit dose contains less than 7 mg, particularly preferably, less than 3.5 mg of Mn-SOD. In the case of pharmaceutical compositions for the treatment of inflammatory diseases of joints, each unit dose preferably contains less than 1.2 mg, particularly preferably less than 0.6 mg (e.g. less than 0.3 mg) of Mn-SOD.

The Mn-SOD to be used according to the invention may occur in all the formations familiar to those

skilled in the art, be they parenteral or enteral or for systemic or topical use, although systemic application is preferred (e.g. i.v., i.m., s.c., i.a., i.p.). The sustained release forms and/or microencapsulated forms (e.g. in liposomes) known to those skilled in the art may well be used for the purposes according to the invention.

In order to prepare the particular preparations, the excipients and carriers known in the art are used, e.g. NaCl, Na-phosphate, hydroxypropyl-ß-cyclodextrin (HPBCD), mannitol and hydroxyethylcellulose.

For systemic treatment, Mn-SOD, preferably the recombinant Mn-SOD, according to EP-A 282 899, may be administered either as a bolus or in a single dose at the dosage according to the invention, to or into the diseased tissue (i.m., s.c., i.p.) or into the bloodstream by some other way (i.v., i.a.) or by infusion, whilst the preparation may be given once or several times a day or on successive days, weekly or monthly depending on the nature, severity and response rate of the disease.

LEGENDS TO THE FIGURES

Fig. 1: Dosage reaction of (a) rh Mn-SOD and (b) Cu/Zn-SOD in oedemas in an FCA-mediated paw model

a: The roman numerals represent those animals treated with various doses of rh Mn-SOD with intraperitoneal (i.p.) route (KG=body weight) I= 50 µg/kg KG, II= 100 µg/kg KG, III= 200 µg/kg KG, IV= 400 µg/kg KG, V= 800 µg/kg KG, VI= 2 mg/kg KG. Each value shows the average data from 6 animals (after 4 hours and 24 hours) and is expressed as a percentage change compared with the placebo [phosphate buffer] (0%)
*= p<0.05, **= p<0.01, ***= p< 0.001 as significant differences from the placebo group (unpaired Student t-Test).

b: The symbols represent the animals treated intraperitoneally (i.p.) with solvent [phosphate buffer] (□) or with Cu/Zn-SOD at a dosage of 100 µg/kg (■). Each dot shows the average data from six animals and the vertical bars indicate the standard deviation.

Fig. 2: Dosage reaction of (a) rh Mn-SOD and (b) Cu/Zn-SOD in joint oedemas in a carrageenan-induced synovitis model.

a: The symbols represent the animals treated by intra-articular (i.a.) route with solvent [physiological NaCl-solution] (□), or rh Mn-SOD in a dosage of 5 µg (■),

12.5 μg (Δ), 25 μg (▲) or 50 μg (X). Each dot indicates the data averaged out from six or seven animals; the vertical bars indicate the standard deviation.

b: The symbols represent the animals treated by intra-articular (i.a.) route with solvent [physiological NaCl-solution] (□), or Cu/Zn-SOD at a dosage of 5 μg (■), or 50 μg (X). Each dot indicates the data averaged out from five or six animals; the vertical bars indicate the standard deviation. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, as significant differences from the animals injected with solvent (unpaired Student T-test).

The Examples which follow are intended to illustrate the invention. All the results are shown as an average + standard errors of the average values (standard deviation). Significance analysis was calculated according to the unpaired Student T-test. A probability of 0.05 was established as the minimum significance level.

Example 1: Adjuvant-induced oedema in the rats paw

Paw oedemas were induced in groups of six male wistar rats (150 to 200 g) by a single subplantare injection (50 µl) of Freund's Complete Adjuvant (FCA, heat-inactivated micobacterium tuberculosis, human strains, C, DT and PN mixed together [Central Veterinary Laboratories, MAFF, Weybridge, Surrey, U.K., freeze-dried] suspended in liquid paraffin BP/10mg/ml). The rats had been immunised six days earlier with 75 µl FCA, administered to the back of the neck. Human recombinant manganese superoxide dismutase (rh Mn-SOD) with a specific activity of 3625 U/mg of protein (prepared according to EP-A 282 899 and obtained from Bender & Co. GesmbH, Vienna) was dissolved in phosphate buffer (0.06M, pH7.8) in final concentrations of 50,100,200,400,800 µg/kg and 2 mg/kg and administered one hour before the FCA induction into a paw in one group of rats (n=6) by intraperitoneal route.

An additional group of rats (n=6) were given Cu/Zn-SOD (from human erythrocytes, specific activity 3610 U/mg Protein, Sigma Chemical Company, U.K.), dissolved in phosphate buffer as described above (100 µg/kg of body weight) one hour before the FCA induction. The paw oedemas were assessed by measuring the circumference of the paw (in mm) 4 hours and 24 hours after FCA induction and the results were expressed as a difference in size between the inoculated paw and uninoculated paw.

Using this model of a cell-mediated hypersensitivity reaction as an example, the dosage-reaction effect of rh Mn-SOD was investigated in oedemas which developed 4 hours and 24 hours after FCA induction. After FCA induction, the paw oedemas developed rapidly within the first 4 hours and reached their maximum after 24 hours. Figure 1a shows the complete series of experiments carried out, in which the effects of rh Mn-SOD were calculated as a percentage

change from the placebo. Pre-treatment with rh Mn-SOD resulted in a "parabolic" dosage-reaction effect, in which higher rh Mn-SOD doses (400-800 µg/kg and 2 mg/kg) had a pro-inflammatory reaction both after 4 hours and also to a lesser extent after 24 hours. A significant reduction in oedema was detected at the lower dosage range of rh Mn-SOD (50,100,200 µg/kg) with average reduction values after 4 hours of 43% ($p < 0.001$) or 25% ($p < 0.01$) and 43% ($p < 0.001$) and 19% ($p < 0.001$) at the two lower rh Mn-SOD doses (50,100 µg/kg after 24 hours. In the groups treated with Cu/Zn-SOD, no significant change could be detected in the development of the oedemas (Figure 1b).

Example 2: Carrageenan-induced synovitis model in the rat

An inflammation was induced in the knee joint of groups of six to seven male wistar rats (150 to 200 g) by a single intra-articular injection (50 µl) of carrageenan (1% w/v in sterile physiological saline solution; carrageenan viscarin 402 made by Marine Colloids Inc., Springfield, USA). At specific times after induction (0, 4, 24, 48 hours) the joint oedemas were assessed by measurement of the width of the knee (mm) and were expressed as the difference between the injected knee joint and the contralateral non-injected knee joint. Groups of rats (n=6-7) were given a single intra-articular injection (50 µl), into one knee joint, of either a) carrageenan, b) carrageenan + rh Mn-SOD (5, 12.5, 25, 50 µg; activity, solution and origin of the rh Mn-SOD as specified in Example 1), c) rh Mn-SOD (50 µg), d) carrageenan + Cu/Zn-SOD (5.50 µg; activity, solution and origin of Cu/Zn-SOD as specified under Example 1) or e) saline (physiological saline solution).

The intra-articular (i.a.) injection (50 µl) of 1% w/v carrageenan into a knee joint in rats resulted in a uniform increase in the joint oedemas which reached its maximum after 24 hours. Treatment with rh Mn-SOD in doses of 25 and 50 µg (i.a.) resulted in marked exacerbation of the oedema reaction up to 48 hours after induction (Figure 2a). A significant reduction in oedema formation (44%, $p < 0.05$) was only achieved with the lower dose of rh Mn-SOD (5 µg) at the 24 hour stage. There were no unfavourable effects on the circumference of the joint after the administration of either rh Mn-SOD (50 µg) or saline on its own.

However, in the group treated with Cu/Zn-SOD up to 48 hours after injection, a significant pro-inflammatory effect was observed.

Example 3: Mn-SOD formulations

The following solutions are suitable for the use according to the invention of Mn-SOD, e.g. for i.v., i.m., s.c., i.a., i.p., enema and topical applications:

| | | | |
|---|---|---|---|
| 1. | MnSOD | 0.1 - 20 | mg/ml |
| | NaCl | 150 | mM |
| | Na-Phosph. | 10 | mM |
| | pH | 5.8 - 7.5 | |
| 2. | MnSOD | 0.1 - 20 | mg/ml |
| | HPBCD | 6 | % |
| | Na-Phosph. | 10 | mM |
| | pH | 5.8 - 7.5 | |

The following formulation is particularly suitable for enemas or topical use:

3. MnSOD (0.15 - 3 mg/ml) in hydrogel (1.75% hydroxyethylcellulose, 0.2% methylparaben as preservatives).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treatment of a human or animal patient suffering from or susceptible to a disease or condition which is caused, partly caused or triggered by oxygen free radicals which comprises administering to the patient 1 to 200 μg of Mn-SOD per kg of bodyweight per day.

2. A method as claimed in claim 1 wherein 1 to 100 μg of Mn-SOD per kg of bodyweight per day is administered.

3. A method as claimed in claim 2 wherein 1 to 50 μg of Mn-SOD per kg of bodyweight per day is administered.

4. A method as claimed in any one of the preceding claims for the treatment of tissue damage.

5. A method as claimed in any one of claims 1 to 3 for the treatment of inflammatory processes.

6. A method as claimed in any one of claims 1 to 3 for the treatment of tissues or organs following transplantation.

7. A method of treatment of a human or animal patient suffering from or susceptible to an inflammatory disease in a joint which comprises administering to the patient 1 to 20 μg of Mn-SOD per 100 μl of the volume of the joint.

8. A method as claimed in claim 7 wherein 1 to 10 μg of Mn-SOD per 100 μl of the volume of the joint is administered to the patient.

9. A method as claimed in claim 8 wherein 1 to 5 μg of Mn-SOD per 100 μl of the volume of the joint is

administered to the patient.

10. A method as claimed in any one of claims 1 to 9 wherein the Mn-SOD is administered systemically.

11. A method as claimed in any one of the preceding claim wherein the Mn-SOD is administered in sustained release form.

12. A method as claimed in claim 1 or claim 7 substantially as herein described.

13. A method substantially as herein described with reference to the Examples.

14. A pharmaceutical composition in unit dosage form comprising as active ingredient Mn-SOD in association with one or more pharmaceutically acceptable carriers or excipients, wherein each unit dose contains less than 14 mg of Mn-SOD when used to treat a human or animal patient suffering from or susceptible to a disease or condition which is caused, partly caused or triggered by oxygen free radicals.

15. A pharmaceutical composition as claimed in claim 14 wherein each unit dose contains less than 7 mg of Mn-SOD.

16. A pharmaceutical composition as claimed in claim 14 wherein each dose contains less than 3.5 mg of Mn-SOD.

17. A pharmaceutical composition as claimed in claim 14 wherein each dose contains less than 1.2 mg of Mn-SOD.

18. A pharmaceutical composition as claimed in claim 14 wherein each dose contains less than 0.6 mg of Mn-SOD.

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19. A pharmaceutical composition as claimed in claim 14 wherein each dose contains less than 0.3 mg of Mn-SOD.

20. A pharmaceutical composition as claimed in claim 14 substantially as hereinbefore described with reference to any one of the Examples.

DATED this 7th day of January 1997

**Boehringer Ingelheim International GmbH**

By its Patent Attorneys

DAVIES COLLISON CAVE

AUSTRALIAN PATENT OFFICE

27. A pharmaceutical composition as claimed in claim 23 wherein each dose contains less than 0.6 mg of Mn-SOD.

28. A pharmaceutical composition as claimed in claim 23 wherein each dose contains less than 0.3 mg of Mn-SOD.

29. A method as claimed in claim 1 or claim 7 substantially as herein described.

30. A method substantially as herein described with reference to the Examples.

31. A use as claimed in claim 12 or claim 18 substantially as herein described.

32. A use substantially as herein described with reference to the Examples.

33. The steps, features, compositions and compounds disclosed herein or referred to or indicated in the specification and/or claims of this application, individually or collectively, and any and all combinations of any two or more of said steps or features.

DATED this EIGHTH day of SEPTEMBER 1993

Boehringer Ingelheim International GmbH

by DAVIES COLLISON CAVE
Patent Attorneys for the applicant(s)

## Abstract

The present invention relates to the use of pharmaceutical preparations containing manganese superoxide dismutase in a low dose for treating diseases and conditions caused by free oxygen radicals.

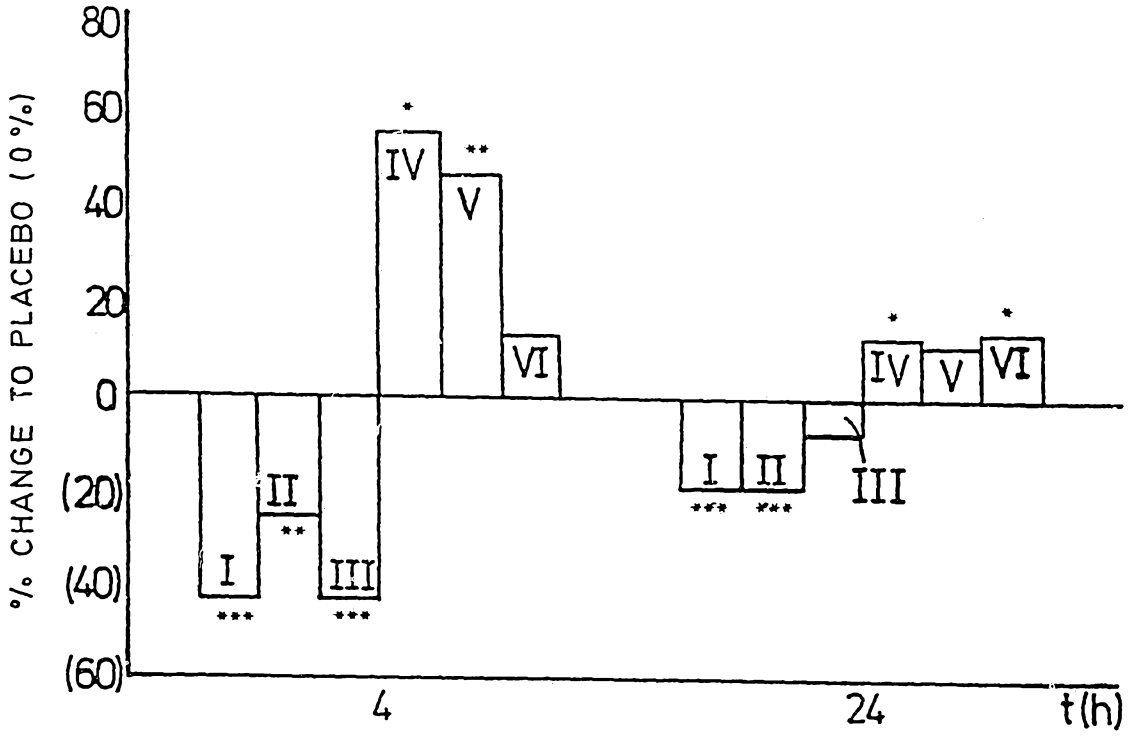
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Fig. 1a

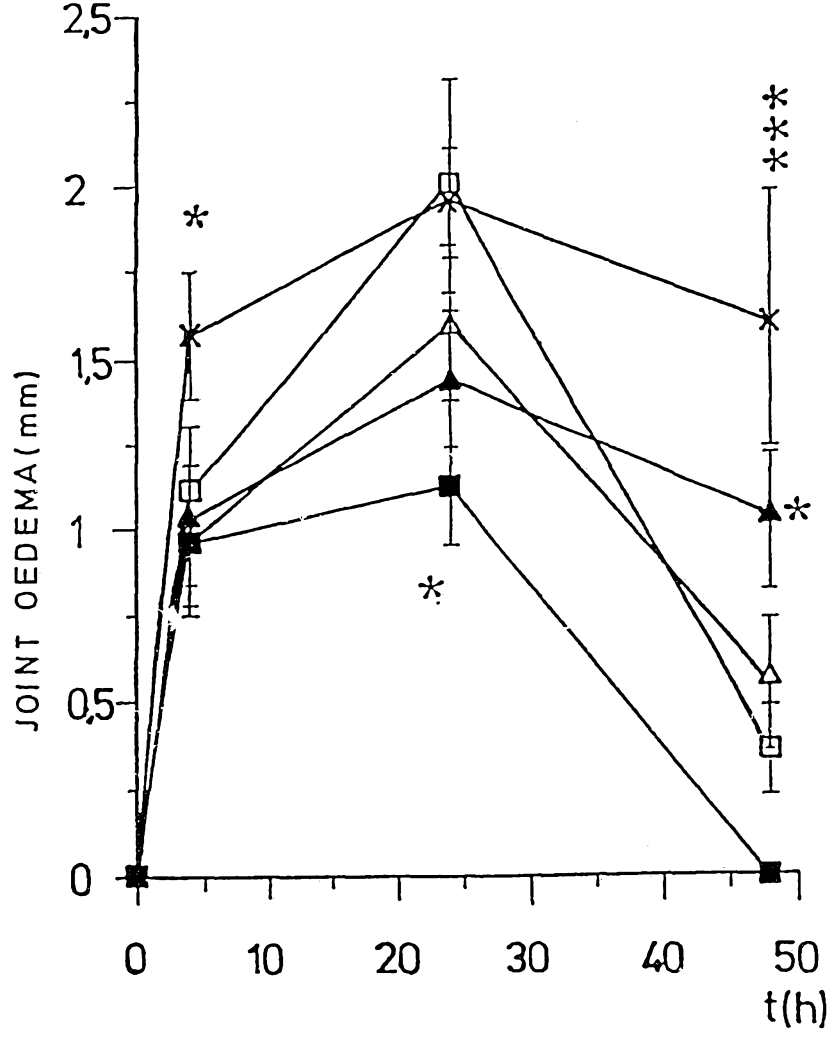
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Fig. 2a

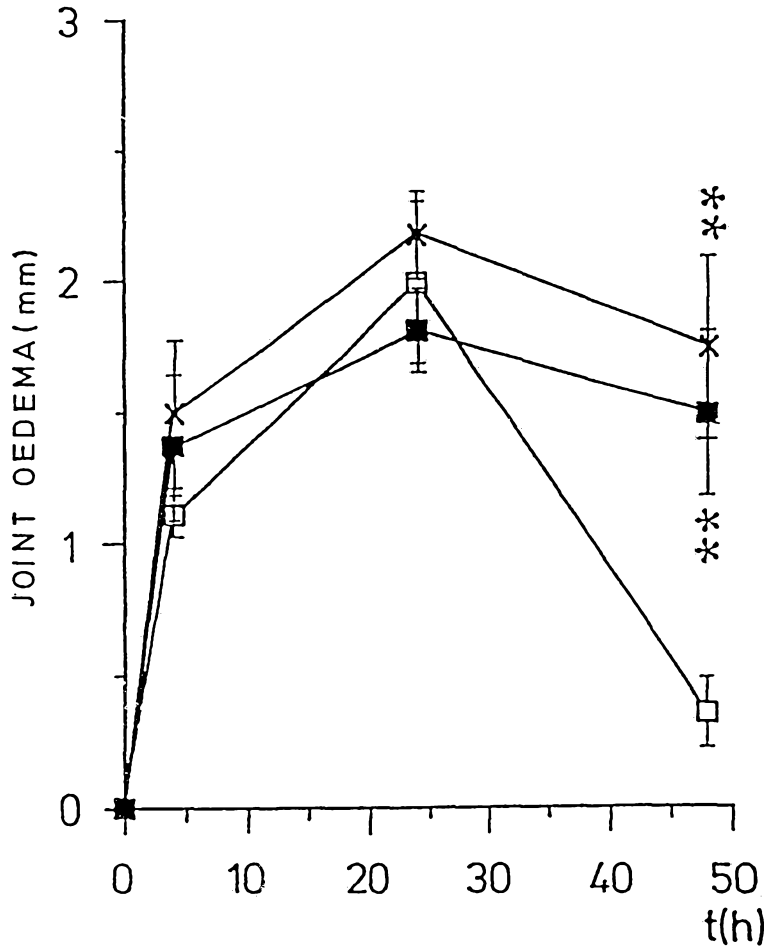
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Fig. 2b