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PROTECTIVE EFFECTS OF HUMAN RECOMBINANT MnSOD IN ADJUVANT ARTHRITIS AND BLEOMYCIN-INDUCED LUNG FIBROSIS

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We have previously shown that human recombinant methionyl manganese superoxide dismutase (MnSOD) is more efficient than CuZnSOD as an anti-inflammatory agent in a model of acute inflammation (Carrageenan-induced paw edema). This effect was attributed to the prolonged half-life of MnSOD in blood ($t_{1/2}$ = 6 h vs. 10 min, respectively). In the present study, the two enzymes were compared in terms of their effectiveness in two systems: (1) Adjuvant-induced arthritis in rats, which is considered to be a model for the chronic situation of rheumatoid arthritis and (2) Bleomycin-induced lung fibrosis, which is a chronic situation believed to be mediated by oxygen free radicals.

Rats inflicted with adjuvant arthritis were treated during the period of maximal joint swelling (Days 15–21 after adjuvant injection) with MnSOD or CuZnSOD (12 to 50 mg/kg, i.p. daily). MnSOD administration resulted in a 50–75% reduction of paw swelling, as well as inhibition of the elevation of serum globulins. A similar treatment with CuZnSOD gave merely marginal effects.

In the second system, lung fibrosis was induced in rats by intratracheal administration of bleomycin. MnSOD (50 mg/kg, s.c.), administered 2 h before and then 2 and 4 days after bleomycin, markedly inhibited lung fibrosis, as evident from lung weight and collagen content measured by the 3rd week. By contrast, CuZnSOD administration did not give a significant effect. The results indicate that MnSOD is superior to CuZnSOD in the treatment of chronic inflammatory processes. In addition, they lend further support to the involvement of oxygen free radicals in bleomycin toxicity.

KEY WORDS: Superoxide dismutase, adjuvant arthritis, bleomycin, lung fibrosis, inflammation.

INTRODUCTION

It is widely accepted that superoxide radicals are involved in a variety of pathological situations leading to tissue damage, such as ischemia-reperfusion injury, inflammatory diseases, chemical toxicity and radiation damage. The metalloenzymes of the superoxide dismutase family have been suggested as therapeutic agents for treating such situations. In fact, bovine and human CuZnSOD were shown to be effective drugs in a variety of animal models and in some clinical situations.

The use of CuZnSOD, however, is limited to acute indications due to the fact that its metabolic half-life is quite short (i.e., about 6 min in the rat). To overcome this drawback, various groups have tried to modify CuZnSOD by its chemical attachment to larger molecules, such as polyethylene glycol[1] or pyran-copolymer.[2] Such modifications, however, may render the natural protein immunogenic or toxic. An alternative approach is to use the mitochondrial SOD, which contains manganese as the active metal and has a prolonged half-life in the blood. We have successfully cloned, expressed and purified human MnSOD.[3] Using this recombinant enzyme, we have previously shown that it is more efficient than CuZnSOD as an anti-inflammatory

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agent in a model of acute inflammation (carrageenan - induced paw edema in the rat).[4] This effect was attributed to the prolonged half life of MnSOD in blood (about 6 h).[4]

In the present study, we have examined the possible use of MnSOD in two chronic situations. The first situation is arthritis, for which a rat model, namely adjuvant - induced arthritis, is widely utilized for the evaluation of anti-arthritic drugs.[5] Another situation is lung fibrosis, which sometimes develops as a consequence of bleomycin chemotherapy of cancer. This toxic effect of bleomycin, which is frequently fatal, is dose-dependent and thus limits its use.[6] Moreover, the risk of pulmonary fibrosis is increased when the anti-cancer drug is given together with radiation therapy.[6] There is evidence which indicates that oxygen radicals are involved in bleomycin-induced lung injury.[7,8]

The present study demonstrates the effectiveness of MnSOD in rat models for the above situations, and indicates that MnSOD is superior to CuZnSOD for use in chronic diseases.

EXPERIMENTAL

Superoxide Dismutases

Recombinant human CuZnSOD was prepared and purified as described by Hartman *et. al.*[9] Recombinant human methionyl-MnSOD was prepared and purified as described by Beck *et. al.*[3]

The Adjuvant Arthritis Model

Female Lewis rats (120–140 g b.w.; Harlan-Sprague Dawley Inc., Indianopolis, IN.) were injected into the stem of the tail with 0.1 ml of complete Freund's adjuvant containing 1 mg of killed *Mycobacterium tuberculosis*. This treatment induces a severe inflammation of multiple joints accompanied by joint swelling, which reaches a maximum after two weeks.[5] At that time, the animals were divided into groups. The experimental groups received daily intraperitoneal injections of MnSOD or CuZnSOD (12.5–50 mg/Kg; 8 rats/group) from Day 15 to Day 21 after adjuvant treatment. The control group (adjuvant only) was not treated. An additional group of rats was kept without adjuvant or SOD treatment (Intact rats) and served for measurements of basal paw volume and globulin/albumin ratio. All measurements were done on Day 22. The paw volume was measured by a Hg-displacement volumeter (a modification of Ugo-Basile Volumeter, Comerio, Italy). Serum albumin concentrations were measured by a colorimetric method,[10] and globulin concentrations were calculated by subtraction of the albumin values from total serum protein.[11]

The Bleomycin-induced Lung Fibrosis Model

Sprague-Dawley female rats (100–120 g b.w.; Charles River Labs, Boston, Mass) were injected intra-tracheally with 1.5 mg bleomycin in 0.5 ml saline (Bleocin; Nippon Kayaku Co., Ltd., Tokyo, Japan). The experimental groups were given a subcutaneous injection of 50 mg/Kg MnSOD or CuZnSOD 2 h before bleomycin administration, and then two additional injections on Days 2 and 4 after bleomycin. A control group was treated with bleomycin alone (the bleomycin group; cf. Figure 3). Another control group was given an intra-tracheal injection of 0.5 ml saline only (the

so-called "Intact Rats" group). Animals were sacrificed on Day 22 and their lungs excised and homogenized in saline. Portions of the homogenates were subjected to hydrolysis for 16 h at 120°C in 6 N HCl. The hydrolysates were neutralized with NaOH and hydroxy-proline content was determined as described by Woessner.[12]

Statistical analysis was done using Student t-test.

RESULTS AND DISCUSSION

Effect of MnSOD and CuZnSOD in Adjuvant Arthritis

As shown in Figure 1, the treatment of the adjuvant-induced disease with MnSOD markedly reduced joint inflammation, as evident from the dose-dependent reduction in paw swelling ($p < 0.05$ for 12.5 and 25 mg/Kg, and $p < 0.001$ for 50 mg/Kg; see Figure 1). It should be emphasized that the adjuvant-induced disease is a severe situation which is very difficult to moderate; it usually requires nearly toxic doses of non-steroidal anti-inflammatory drugs (such as diclofenac; data not shown) in order to achieve significant effects similar to the MnSOD effects shown here. Hence, these therapeutic effects of MnSOD are impressive. As expected, CuZnSOD caused merely marginal and non-significant effects at the same doses. This difference between the two enzymes is most probably due to the much shorter half-life of CuZnSOD as compared to MnSOD. The anti-inflammatory action of SOD, which is the result of superoxide radical scavenging, is most probably of a local nature, thus occurring at the sites of tissue inflammation. Hence, it would be unexpected to see a systemic effect of SOD on the immunological course of the disease. The results of serum globulin-to-albumin ratios, shown in Figure 2, seem to indicate differently, since MnSOD treatment did attenuate the adjuvant-induced increase in serum globulins. It should be mentioned, though, that this effect did not attain statistical significance, and more data are needed in order to establish this point.

From the above study, MnSOD appears to be a promising therapeutic drug for the

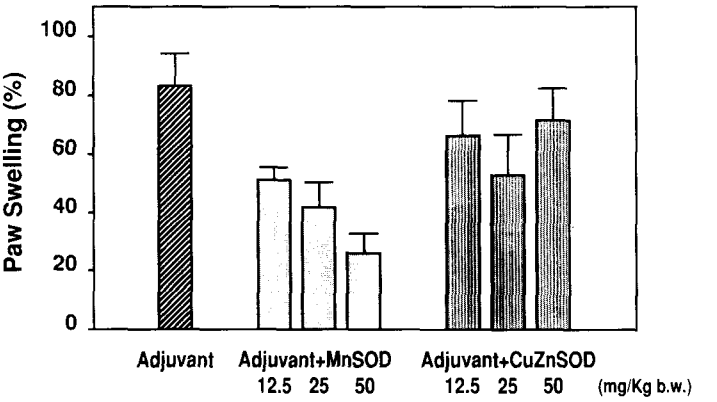

FIGURE 1 Effect of MnSOD and CuZnSOD on paw swelling in rat adjuvant arthritis. Arthritis was induced in Lewis rats (8 rats/group) by Freund's adjuvant injection into the tail stem. Recombinant human MnSOD or recombinant human CuZnSOD were administered daily (12.5, 25 or 50 mg/Kg intra-peritoneally) from Day 15 to Day 21 after adjuvant treatment. Paw volume was determined on Day 22. The bars and vertical brackets represent the percent increase (mean ± S.E.M) in paw volume as compared to the mean paw volume of a group of intact rats at the same age.

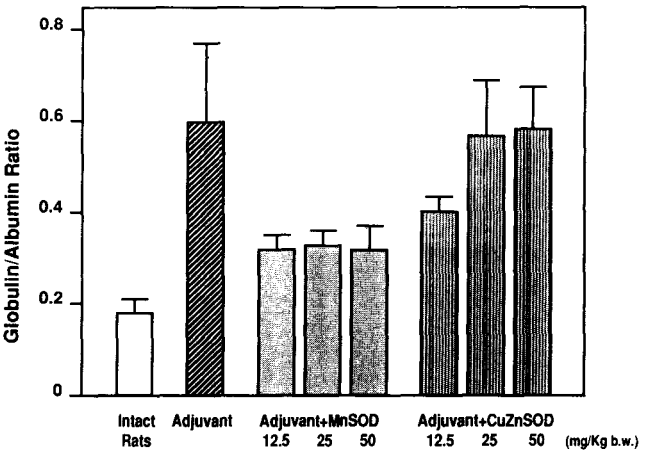

FIGURE 2 Effect of MnSOD and CuZnSOD on serum globulin-to-albumin ratio in rat adjuvant arthritis. For details, see legend to Figure 1 and the Experimental section.

systemic treatment of chronic arthritis. Its major advantages over other drugs are its relatively long half-life in the blood and the expected low toxicity due to its being a naturally-occurring human protein.

Effect of MnSOD on Bleomycin-induced Lung Fibrosis

In the rat model, pulmonary fibrosis induced by bleomycin was clearly indicated by the doubling of lung collagen content (represented as hydroxy-proline; Figure 3).

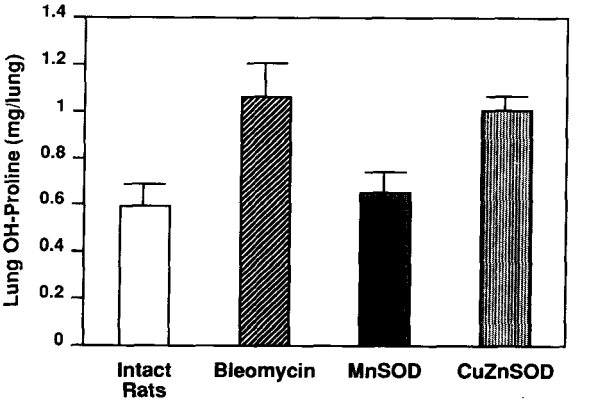

FIGURE 3 Effect of MnSOD and CuZnSOD on bleomycin-induced lung fibrosis in rats. The animals were given intra-tracheal injection of saline (the Intact Rats group; N = 6) or bleomycin (1.5 mg/rat; the Bleomycin, MnSOD and CuZnSOD groups; N = 9-10 rats/group). Human recombinant SOD (50 mg/Kg of MnSOD or CuZnSOD, respectively) was given subcutaneously 2 h before bleomycin, and then two additional injections on Days 2 and 4 after bleomycin. The bars and vertical brackets represent the mean and S.E.M. of the content of hydroxyproline in the lungs on Day 22 after bleomycin treatment. For further details, see the Experimental section.

MnSOD treatment significantly inhibited lung fibrosis (cf. Figure 3), even though the value of lung hydroxy-proline content was still slightly above normal. By contrast, CuZnSOD was not effective.

The ability of MnSOD to inhibit the bleomycin effect in the lung lends support to the postulate that oxygen free radicals are involved in the action of this anti-tumor agent.[7] Thus, it accords well with the observations that bleomycin and hyperoxia produce excessive lung fibrotic changes in a fore-shortened time interval, compared to bleomycin alone[8] and that endotoxin, known to increase the endogenous levels of scavenger enzymes, has been shown to partially inhibit bleomycin-induced lung damage.[13] It is not clear whether the mediation of the bleomycin effect by oxygen radicals is limited to its action in the lung, or whether it is involved as well in the anti-tumor action of this antibiotic agent. This point should be clarified before MnSOD could be offered as a lung potective drug in bleomycin therapy.

In conclusion, MnSOD appears to be an effective, long acting agent for use in a variety of chronic situations that involve oxygen radical action.

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