Topical Treatment with Superoxide Dismutase is Effective for Experimentally Burned Rats

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ABSTRACT. The effect of topical superoxide dismutase (SOD) treatment for experimental burns was investigated in 108 male Wister rats. A deep dermal burn was induced in the dorsal skin with 40 ml of hot water (95°C) under general anesthesia. Daily application of topical SOD for three day after injury resulted in reduced cutaneous inflammation and rapid wound healing. Serum and skin lipid peroxide concentrations were significanty reduced in rats treated with SOD, and the reduction in skin SOD activity after the burn was minimised by topical treatment with SOD. SOD scavenges superoxide and subsequent oxygen by-product, and reduces the lipid peroxide production. Topical application of SOD for the first three days is effective for and a promising approach to the treatment of burns.

Key words: experimental burn — lipid peroxide — superoxide dismutase

Recent research on the biological role of reactive oxygen species in inflammatory diseases and ischaemia-reperfusion injuries has prompted us to use antioxidants in their treatment.[1-3] Experimental thermal injury of the skin causes an appreciable increase in the lipid peroxides in the injured skin, and they leak into the blood stream.[4] Lipid peroxides are partly formed by mediation of reactive oxidants and have a long half life with deleterious effects on living tissues. Superoxide dismutase (SOD), which catalytically scavenges superoxide, suppersses the lipid peroxidation process. In the present study, we investigated the effect of topical treatment with SOD on wound healing and skin SOD activity after experimental burns with simultaneous assays of both serum and skin lipid peroxide concentration.

MATERIAL AND METHODS

Experimental burns

Experimental burns were induced on the dorsal skin of male Wistar rats (250-350g). The hair was shaved under the anaesthesia with pentobarbital sodium (Nembutal), and 40 ml of hot water (95°C) was poured on to the back, which was covered by the gauze. This resulted in a deep dermal burn of 40% of the body surface area.

Topical SOD treatment

A human recombinant Copper, Zinc, -SOD (4000 U/mg weight) preparation in a water soluble base which is mainly composed of Polyethylene glycol was

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applied for three days after the burn. About 10 g of the preparation was used for each rat. The vehicle alone was applied to the controls.

Clinical and histological observatios

Rats were divided into three groups; SOD-treated, vehicle-treated and untreated, (n=6 in each group).

Wounds were observed both macroscopically and histologically until they were fully epithelialised. The percentage of epithelisation was judged daily and biopsy specimens were examined microscopically after staining with haematoxylin and eosin.

Serum and skin lipid peroxide assay

Serum and skin lipid peroxide concentrations were assayed by measuring the thiobarbituric acid method according to Yagi[5] and Ohkawa *et al*[6] and expressed as malondialdehyde (MDA) nmol/mg protein.

Skin SOD activity assay

Skin SOD activity was assessed by its ability to inhibit the xanthine oxidase mediated reduction of cytochrome C as described by Suzuki *et al*.[7]

Statistical analysis

All the values were expressed as mean plus or minus SEM (standard error of mean). Comparison between the means was made using analysis mf variance and the level of significance was settled at 0.05 unless otherwise stated.

RESULTS

Macroscopic findings (Fig 1)

In the untreated group, the wound gradually became covered by crusts three days after burn injury, and after one week, 30% of the wound area had epithelialised. This had increased to 70% by the end of the second week with erosion and ulcers in the rest of the area (Fig 1a). Almost complete epithelisation had taken place by four weeks with partial alopecia. By contrast, there was complete epithelialisation within two weeks without formation of crusts in the SOD-treated group (Fig 1b). In the vehicle-treated group, the epithelisation process was slower than in the SOD-treated group but better than in the untreated group. After two weeks, about 90% of the wounds in vehicle-treated group was covered with epidermis, with spots of erosion and ulcer.

Histological findings (Fig 2)

Microscopic results were similar to the macroscopic results in each group. In the SOD-treated group, both infiltration of inflammatory cells and edema were appreciably reduced compared with the other two groups. In addition, the epithelialisation process was much better organised in the SOD-treated group than in the other two groups.

Changes in serum lipid peroxide concentrations (Fig 3)

In the untreated group, serum lipid peroxide levels peaked three to six

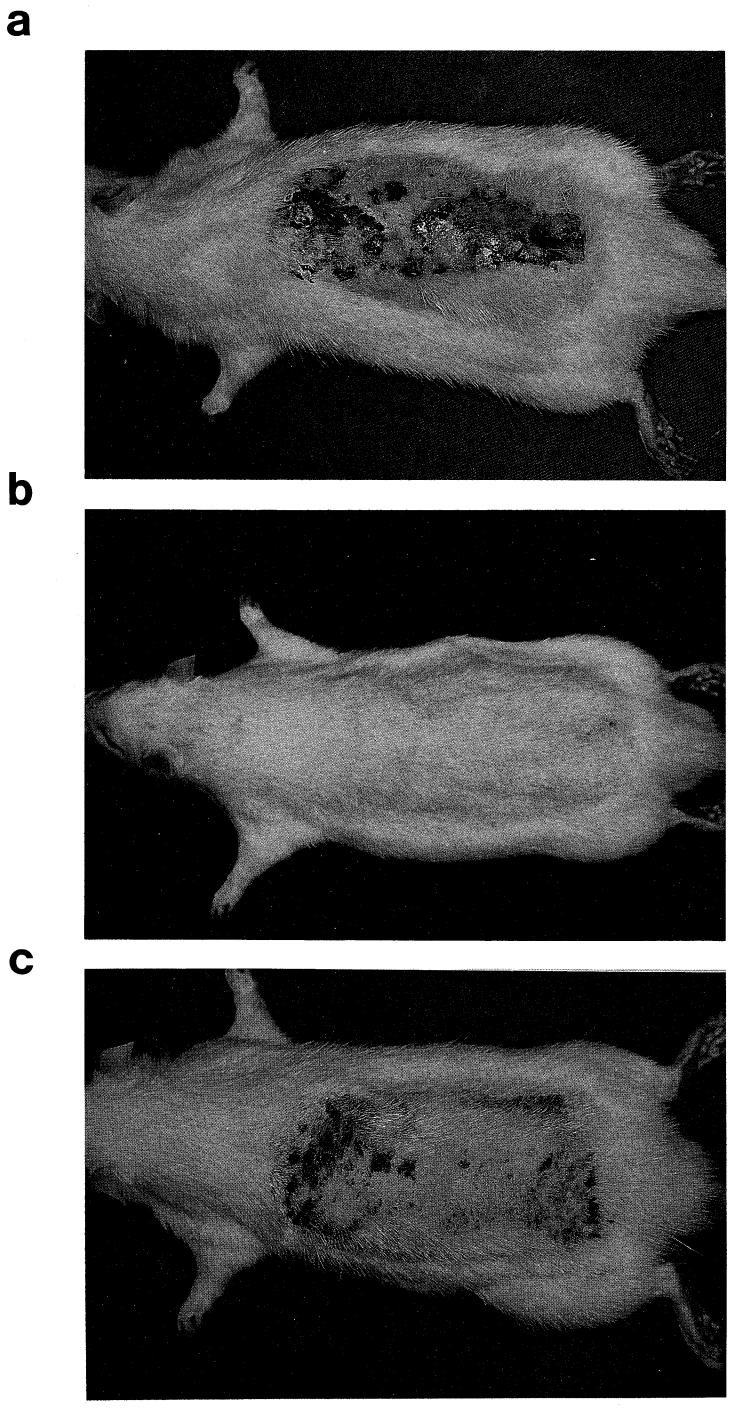

Fig 1. Appearances two weeks after the burn
a: untreated group, about 70% epithelisation with erosion and ulcers over the rest of the area
b: SOD-treated group, prompt epithelisation with no crust formation
c: vehicle-treated group, about 90% epithelisation with spots of ulceration

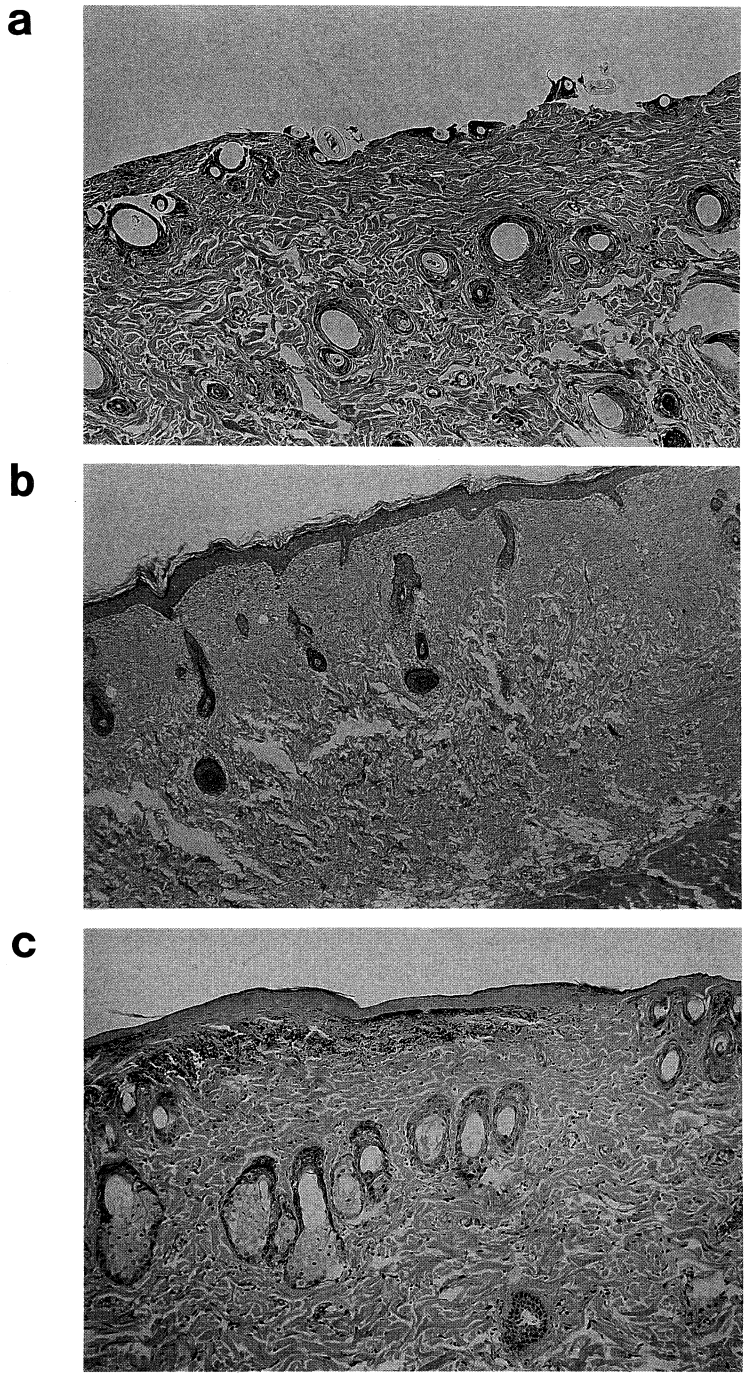

Fig 2. Histological findings one week after the burn injury
a: untreated group, b: SOD-treated group, c: vehicle-treated group
The SOD-treated group showed an appreciable reduction both infiltration of inflammatory cells and edema compared with other two groups.

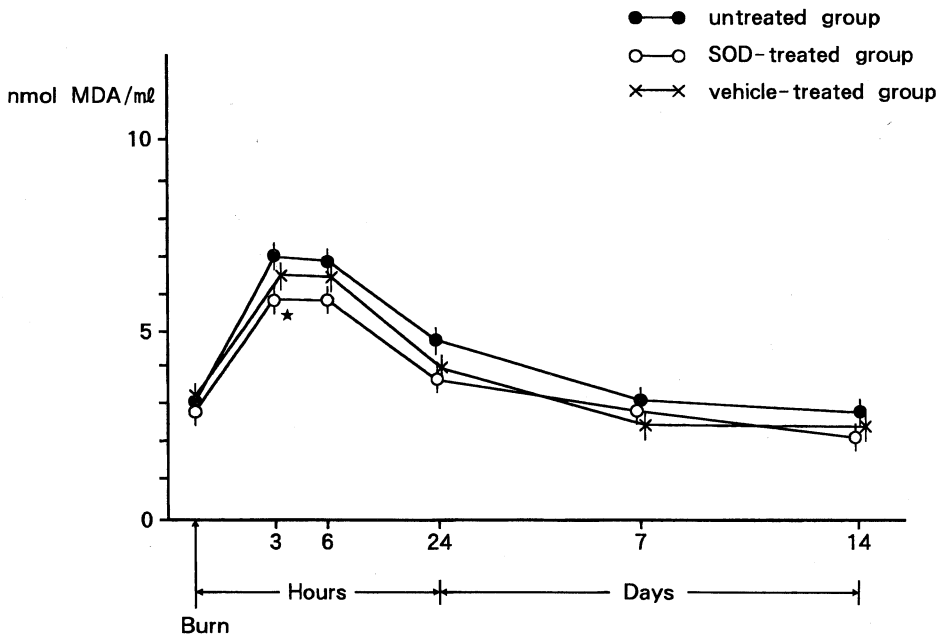

Fig 3. Changes in serum lipid peroxide concentrations after experimental burns in rats expressed as malondialdehyde (MDA) nmol/ml
Satistical significance: *P<0.01 against untreated group

hours after the burn, remained steady for about three hours and then reduced. These changes were similar to those reported by Nishigaki *et al.*[4] In the present experiments, however, the lipid peroxide concentration did not return to baseline until two weeks after the burn. In the SOD-treated group, serum lipid peroxide concentration was significantly reduced ($P<0.01$) and returned to the control level more quickly than those in the untreated group. There was more obvious inhibition of the increase in serum lipid peroxide concentration in the SOD-treated group than in the animals treated with the vehicle alone.

Changes in SOD activity in skin (Fig 4)

Skin SOD activity decreased immediately after burns in the untreated group ($P<0.01$); this change continued for about three days and then increased slowly. In the SOD-treated group, skin SOD activity increased after application, and then returned gradually to the reference range. Decrease of skin SOD activity after burn have inhibited by topical application of SOD. In the vehicle-treated group, the changes in skin SOD activity were similar to those in the untreated group.

Changes in skin lipid peroxide levels (Fig 5)

Skin lipid peroxide concentrations also peaked three hours after the induction of the burns, and gradually returned to the control level by two weeks. Topical SOD treatment effectively inhibited the production of lipid peroxides induced by the experimental burns. ($P<0.001$)

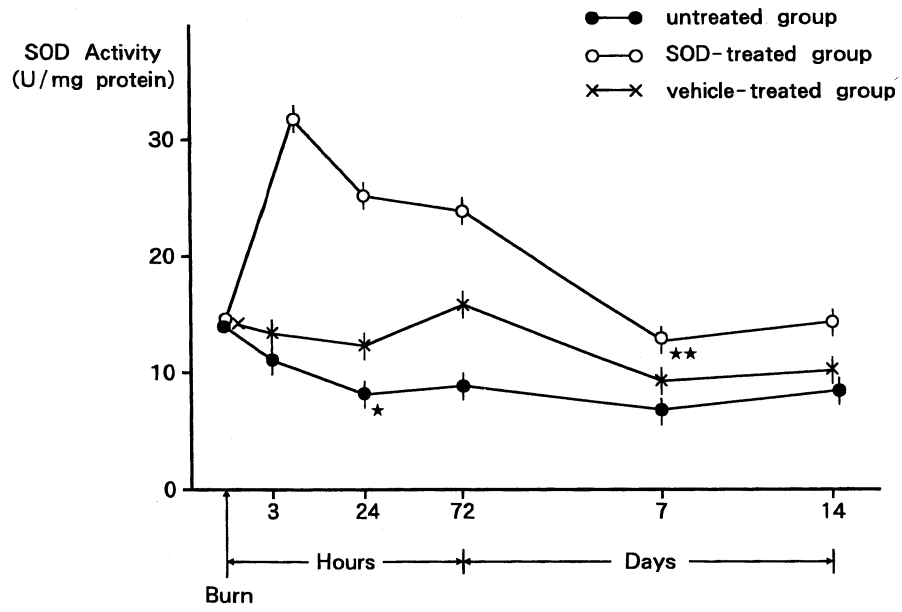

Fig 4. Changes in skin superoxide dismutase activity after experimental burns in rats(U/mg protein)
Satiscal significance: ★P<0.01 against time zero, ★★P<0.05 against untreated group

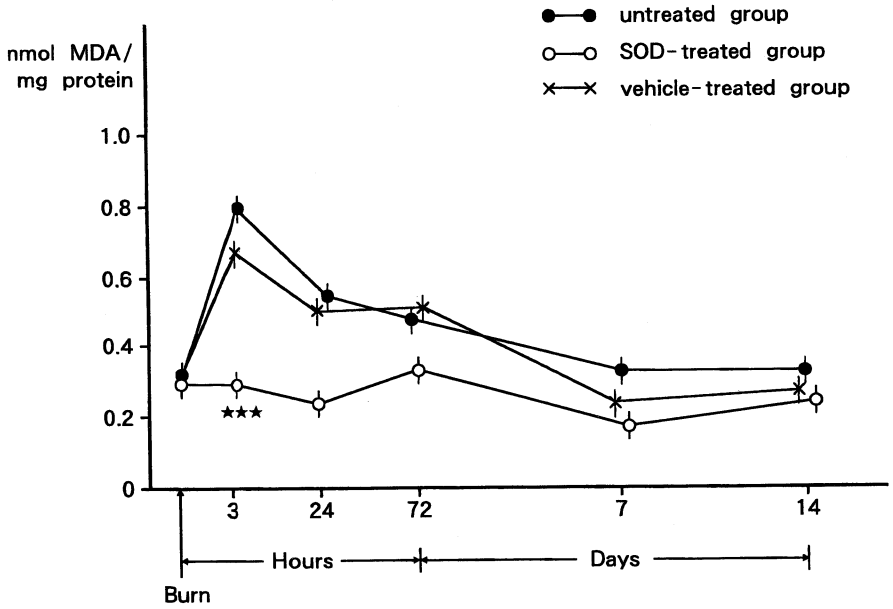

Fig 5. Changes in skin lipid peroxide concentration after experimental burns in rats expressed as malondialdehyde (MDA) nmol/mg protein
Satiscal significance: ★★★P<0.001 against untreated group

DISCUSSION

The lipid peroxidation process is partly related to the production of reactive oxygen species, which are believe du to affect membrane structures by radical reactions.

Peroxidation of cell membrane lipids occurs after burns caused by direct physical injury to the cell membrane by heat.[8] The lipid peroxides induce further tissue damage in combination with secondary tissue injury mediated by inflammatory cells. SOD scavenges superoxide and subsequent oxygen byproducts resulting in the inhibition of lipid peroxide production. Reduced cutaneous SOD activity has been reported after experimental burns in guinea pig.[8] In the present study, skin SOD activity was reduced immediately after experimental burns, and this reduction lasted for three days. In contrast, both serum and skin lipid peroxide levels peaked three hours after burns, and returned to the reference range by two weeks. These changes were effectively inhibited by topical SOD treatment. Yagi reported that the lipid peroxide concentration in burned skin increased significantly, and then the lipid peroxides that had formed in the burned skin leaked into the blood stream, causing the increase in the serum lipid peroxide concentrations.[10] Intravenous infusion of the antioxidant enzyme polyethylene glycol-conjugated superoxide dismutase reduces the plasma concentrations of lipid peroxidation products in burned patients.[11] However, the targed of SOD is skin itself, so the topical application shoud be more reasonable approach. For iujuries to the skin, topical application is a direct and effective way of delivering a drug. As cutaneous barrier function is impaired at the site of the burn antioxidants seem to be absorbed more easily than through the intact skin.[12] In the present experiments, topical application of SOD for the first three days was effective, and a promising approach for the future treatment of burns. There is increasing experimental evidence to suggest that antioxidant treatment is effective after thermal injury.[13,14] We think that the initial suppression of peroxidation in the skin may protect against further tissue injury.

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