Effects of Local Hyperthermia Treatment on Skin SOD Activity in Mice

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Summary The effect of local hyperthermia treatment (43°C, 45 min) on normal mouse skin was studied by examining superoxide dismutase (SOD) activity and histology of the skin. SOD activity of the skin decreased significantly 1, 4, 7, and 14 days after the treatment. The decrease reached a peak 4 days after the treatment and recovered to some extent by 14 days after the treatment. The decrease in SOD activity may be due to consumption of SOD by inflammation as well as to the destruction of SOD by hyperthermia. Histological examination of hyperthermia-treated skin showed edema, bleeding, and inflammatory cell infiltration in the dermis, and individual cell keratinization and acanthosis in the epidermis. It is important to estimate the decrease in SOD activity and tissue injury of the skin by hyperthermia in order to use hyperthermia more effectively with fewer side effects.

Key Words: hyperthermia, histology, superoxide dismutase, skin, mice

Hyperthermia has been successfully used in the treatment of cancer [1, 2], and the importance of hyperthermia treatment has been increasing with the improvement of apparatus as well as the elucidation of the mechanisms of hyperthermia treatment. Hyperthermia has been also used in the treatment of dermatological diseases such as psoriasis vulgaris [3] and sporotrichosis [4]. However, skin damage induced by hyperthermia treatment has not been studied extensively.

Recently it was reported that reactive oxygen species (ROS) may be involved in the cytotoxic mechanism of hyperthermia [5], and that the lipid peroxide level was demonstrated to increase after thermal injury in rats [6]. These reports suggest that ROS and lipid peroxides play roles in skin damage induced by hyperthermia treatment.

In this study, we examined superoxide dismutase (SOD) activity of the skin of

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mice before/after hyperthermia treatment in order to estimate quantitatively the host's defense mechanism against oxidative tissue injury of the skin induced by hyperthermia treatment, because SOD is a scavenger of ROS and one of the parameters of the host's defense mechanism against oxidative tissue injury. In addition, we investigated histologically the time course of skin damage induced by hyperthermia treatment.

MATERIALS AND METHODS

Animals. Albino female ICR mice, 8 to 10 weeks old, were used. All mice used in experiments were obtained from Shimizu Laboratory Supplies, Kyoto.

Local hyperthermia treatment. Local hyperthermia treatment was given according to the method of Miyakoshi et al. [7, 8] with slight modification. As shown in Fig. 1, each unanesthetized mouse was put into a special cage. The left hind leg was strapped down with surgical tape, and a 50 g weight was attached to the right hind leg with an adhesive (Toa Chemical Co., Tokyo) to prevent the mouse from withdrawing the leg when it was put into hot water. The leg was immersed in a circulating water bath (ET-35P; Toyo Seisakusho, Tokyo), in which the temperature of the hot water was maintained constant within the desired temperature ±0.05°C. During hyperthermia treatment, a current of cool air was blown across the bath to prevent the body temperature from rising and to reduce the humidity of the inspired air. In our experiments, hyperthermia treatment at 43°C for 45 min was given to each mouse.

Histology of the skin. Biopsy specimens were obtained from hyperthermia-treated skin (the right hind leg) and non-treated skin (the left hind leg) of each mouse 1 and 3 h, 1, 2, 3, 7, and 14 days after hyperthermia treatment. They were then fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin. Histologically we observed and scored the extents of edema, bleeding, dilatation of blood vessels, the infiltration of inflammatory cells, acanthosis, and presence of individual cell keratinization. Observations were made and scoring was done on the histology of the non-treated skin of the same animal as a control.

SOD activity of the skin. Skin specimens were obtained from hyperthermia-treated and non-treated skin 1, 4, 7, and 14 days after the treatment. Biopsy specimens, each weighing 1.5–2.0 g, 2–3 mm thick, and containing both dermis and epidermis, were dissected, finely minced with scissors, and homogenized. Then, they were sonified, suspended in Krebs-Ringer phosphate buffer, and centrifuged. Superoxide anion (O$_2^-$) was generated by the xanthine and xanthine oxidase (XOD) system. Fifty microliters of 0.66 mM ferricytochrome c, 0.1 ml of 2 mM hypoxanthine (HPX) in physiological saline, 0.05 ml of 50 mM EDTA and physiological saline were mixed in total volume of 2.3 ml, and 1 ml of SOD-containing fluid was added. Finally, 0.1 ml of dialyzed XOD at a concentration of 0.12 U/ml was added to generate O$_2^-$. Under these conditions, the amount of SOD activity

Fig. 1. Procedure for hyperthermia treatment. An unanesthetized mouse was put into a special cage, after which the left hind leg was strapped down with surgical tape, and a 50 g weight was attached to the right hind leg with an adhesive to prevent the mouse from withdrawing the leg when it was put into hot water.

Fig. 2. Histology 1 h after hyperthermia treatment. Edema and bleeding were remarkable in the upper dermis (H.E. stain, ×100).

Fig. 3. Histology 3 days after the treatment. Dense infiltrates of mononuclear cells were observed in the dermis (H.E. stain, ×100).
required to inhibit the rate of reduction of cytochrome $c$ by 50% (i.e., a rate of 0.0125 absorbance per min) was defined as 1 unit of activity [9]. In the SOD activity assay of the skin specimens, the samples not only inhibited the cytochrome $c$ reduction but also directly reduced a small amount of cytochrome $c$ without mediation of $O_2^-$ [10]. Therefore, the actual value of SOD activity induced by the skin homogenates themselves was calculated by taking the amount of cytochrome $c$ directly reduced by the skin homogenates themselves into consideration according to the formula of Niwa et al. [11]. Since percent inhibition did not parallel the real SOD activity above approximately 1 unit, the activity was adjusted according to Asada et al.’s formula [12]. The protein content of each suspension was assayed by Lowry et al.’s method [13]. SOD activity was expressed as units/mg protein.

Fig. 4. Histology 7 days after the treatment. Acanthosis was remarkable (H.E. stain, ×200).

Fig. 5. Histology 14 days after the treatment. Individual cell keratinization could be seen (arrow). Acanthosis was also observed (H.E. stain, ×200).

RESULTS

Histological changes of the skin after hyperthermia treatment

Results are summarized in Table 1. At an early stage such as 1 and 3 h after the treatment, no remarkable changes were observed in the epidermis, however, edema, and bleeding were remarkable in the upper dermis (Fig. 2). Inflammatory cell infiltrates, mainly consisting of polymorphonuclear leukocytes (PMNs), were observed in the dermis. One and three days after the treatment, edema and bleeding of the upper dermis decreased gradually, and the more remarkable inflammatory cell infiltration was observed with the infiltrates mainly consisting of mononuclear cells (Fig. 3). In the epidermis, some cases showed slight acanthosis or individual cell keratinization. Seven days after the treatment, dermal changes such as edema and bleeding disappeared; however, inflammatory cell infiltration was still observed. In the epidermis, some cases showed remarkable acanthosis (Fig. 4) and individual cell keratinization. By 14 days after the treatment, the dermis had returned normal, however, acanthosis and individual cell keratinization were still observed (Fig. 5). Collagen fibers and elastic fibers were not damaged remarkably throughout the observation period.

<table>
<thead>
<tr>
<th>Table 1. Histological changes after hyperthermia treatment.*</th>
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<td></td>
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<tr>
<td>Epidermal changes</td>
</tr>
<tr>
<td>Acanthosis</td>
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<td>Individual cell keratinization</td>
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<td>Dermal changes</td>
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<td>Edema</td>
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<td>Bleeding</td>
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<td>Dilatation of blood vessels</td>
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<td>Cell infiltration</td>
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<td>*Local hyperthermia was given at 43°C for 45 min.</td>
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Table 2. SOD activity of the skin after hyperthermia treatment.*

<table>
<thead>
<tr>
<th>Days after treatment (days)</th>
<th>SOD activity in treated skin (units/mg protein)</th>
<th>SOD activity in non-treated skin (units/mg protein)</th>
<th>p value**</th>
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<tr>
<td>0***</td>
<td>—</td>
<td>11.28 ± 1.58</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>3.40 ± 1.04</td>
<td>13.83 ± 1.34</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>4</td>
<td>1.69 ± 0.62</td>
<td>9.08 ± 1.06</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>7</td>
<td>3.12 ± 1.99</td>
<td>12.28 ± 2.12</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>14</td>
<td>8.20 ± 0.92</td>
<td>12.10 ± 1.26</td>
<td>&lt;0.01</td>
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*Local hyperthermia was given at 43°C for 45 min. **Statistical analysis was performed by Student’s t-test. ***Prior to treatment.

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SOD activity of the skin before/after hyperthermia treatment

Results are shown in Table 2. After hyperthermia treatment, SOD activity of the treated skin was always significantly lower than that of normal skin throughout the observation period. The decrease in SOD activity of hyperthermia-treated skin reached a peak 4 days after the treatment, and some recovery was noted 14 days after the treatment. But the activity at that time was still significantly lower than that of normal control skin.

DISCUSSION

Histological changes in the skin induced by local hyperthermia treatment could be divided into early changes which were observed 1–3 h after the treatment and late changes observed around 7 days after the treatment. One, three, and 24 h after the treatment, no remarkable changes were observed in the epidermis; however, severe edema and bleeding were seen in the dermis. Three and seven days after the treatment, acanthosis and individual cell keratinization were often observed, while edema and bleeding in the dermis had disappeared by that time. Inflammatory cell infiltrates in the dermis mainly consisted of PMNs 1 and 3 h after the treatment, but of mononuclear cells 1, 3, 7, and 14 days after the treatment.

The decrease in skin SOD activity reached a peak 4 days after the treatment and recovered to some extent by 14 days after it. This decrease in skin SOD activity may be due to consumption of SOD by inflammation where PMNs are infiltrating in its early phase and may play an important role, additionally some SOD may be inactivated by hyperthermia. In this hyperthermia-induced inflammation, it is possible that ROS are produced by infiltrating PMNs with resultant tissue injury, and the edema observed 1–3 h after the treatment may indicate the existence of ischemia produced by disturbed local circulation. It is also probable that lipid peroxide is produced by heat and induces tissue injury, because it was reported that the lipid peroxide level of the skin increased after thermal injury in rats [6]. And Gi showed the possibility that ROS are involved in the cytotoxic mechanism of hyperthermia [5]. Taking these possibilities and our data into consideration, we conclude that local hyperthermia treatment induces tissue injury of normal skin and that ROS might be involved in this skin tissue injury.

Hyperthermia will be more frequently used as it becomes more widely recognized and it will often be applied to the skin. Therefore, we must accumulate more knowledge about the unfavorable side effects of hyperthermia treatment and find means of reducing them.

REFERENCES


