Hydrogen Peroxide-Induced Dermatitis in WBN/Kob-Ht Rats

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Abstract: Light and electron microscopic examinations were carried out on the dorsal skin to which hydrogen peroxide (HPO) (3%, 6%, and 10%) was topically applied for 7 consecutive days in Wistar rat-derived inbred WBN/Kob-Ht rats which have an autosomal dominant gene responsible for their characteristics of hypotrichosis. In addition to focal epidermal thickening, keratinocyte necrosis, dermal mononuclear cell infiltration and focal detachment of the epidermis from the dermis by fluid-filled spaces were detected. This is thought to be brought about by edema due to prominent capillary endothelial damage in the superficial dermis. The damage to keratinocytes and capillary endothelial cells was thought to be induced by HPO itself and free radicals generated by HPO. In addition, these changes were apparently more severe in WBN/Kob-Ht rats than in Wistar rats used as controls.

Key words: dermatitis, hydrogen peroxide, WBN/Kob-Ht rat

Both the quality and quantity of environmental chemicals surrounding us continue to increase year by year, and the skin is often exposed to such environmental chemicals because it is one of the largest organs in mammals [4], but there are a few reports of dermatitis induced by environmental chemicals.

Hairless or hypotrichotic animals are expected to be suitable experimental animals for dermatological investigations, especially for a long-term dermatotoxicological study, because they do not require shaving which probably affects the skin physiology.

Kimura and Doi [3] have established a colony of laboratory hairless dogs derived from Mexican hairless dogs. The skin conditions of laboratory hairless dogs are similar to those of humans [2], but the handling and care of dogs are more troublesome than those of laboratory rodents. In this regard, rats have an appropriate body size and are actually used in various toxicity studies including dermatotoxicity.

A Wistar-derived inbred strain of hypotrichotic rats named WBN/Kob-Ht, which has an autosomal dominant gene (Ht: dominant hypotrichosis) responsible for their characteristics of hypotrichosis, has been developed in Japan [6]. WBN/Kob-Ht rats (HtRs) have sparse downy hair on the head, dorsum and extremities throughout their life span (Fig. 1). Except for the hair follicles being less well developed and situated in more superficial dermis in HtRs, the skin histology of HtRs is similar to that of hairless rats. Detailed histological and ultrastructural characteristics of the dorsal skin of
HtRs will be reported elsewhere.

As the first step in evaluating the usefulness of HtRs in the dermatotoxicity study on environmental chemicals, histopathological and ultrastructural examinations were carried out on the skin over the dorsum of HtRs to which hydrogen peroxide (HPO), one of the important environmental chemicals, was applied.

Five 7-week-old male HtRs were used. In addition, 5 age-matched male Wistar rats (WRs) were also used as controls. The rats were purchased from Saitama Experimental Animal Supply Co. (Saitama). They were individually kept in isolater cages (Niki Shoji Co., Tokyo) under controlled conditions (temperature, 23 ± 2°C; relative humidity, 55 ± 5%) and fed pelleted diet, MF (Oriental Yeast Co., Tokyo) and tap water ad libitum.

To the rats were HPO solution (3, 6 and 10%) was topically applied. Mesh patches for human skin tests (Patchtester, Trii Medical Co., Tokyo) containing 0.04 ml of HPO solution of each concentration or distilled water were applied to 4 points on the dorsal skin for 7 consecutive days. Prior to the application, the WRs were shaved with surgical clippers (Natsume Co., Tokyo). The patches were changed every day. All the rats were sacrificed by exanguination under ether anesthesia 1 day after the last HPO-application.

Skin samples were fixed in a commercially available fixative (18.5% formaldehyde in methanol) (Yufix, Sakura Seiki Co., Tokyo) and four-μm paraffin sections were stained with hematoxylin and eosin (HE) for histopathological examination. For electron microscopical examination, small pieces of the skin samples were fixed in 2% glutaraldehyde in phosphate buffer (PB) (pH 7.2), post-fixed in 1% osmium tetroxide in the same buffer and then embedded in epoxy resin (Epon812, Oken Shoji Co., Tokyo). Ultrathin sections were double-stained with uranil acetate and lead citrate and observed under an electron microscope, JEOL-1200EX (JEOL, Tokyo).

In the dorsal skin of HtRs treated with 3% HPO, mild and focal epidermal thickening was observed. At and around such thickened epidermis, keratinocytes showing signs of pyknosis and/or intracytoplasmic edema were sporadically found especially in the basal layer (Fig. 2), and mild infiltration of mononuclear cells was sometimes observed in the superficial dermis (Fig. 3). In the deeper dermis, an increase in the number of mast cells was found. In the dorsal skin of HtRs treated with 6% HPO, the above mentioned changes progressed and the border between the epidermis and the dermis became irregular (Fig. 4). In some portions, the epidermis was partially detached from the dermis, leaving a space filled with fluid between them (Fig. 5). In the dorsal skin treated with 10% HPO, focal trans-epidermal necrosis was observed in some cases. Although apparently less severe, skin lesions of a similar histopathological nature to those in HtRs were detected in WRs (Fig. 6).

Similar histopathological changes to those mentioned above were reported in the dorsal skin of Sencar mice which received single applications of HPO at higher

Fig. 1. Appearance of WR (left) and HtR (right).

Fig. 2. Dorsal skin of a HtR treated with 3% HPO. Epidermal thickening with a small number of keratinocytes (arrowheads) showing pyknosis and/or intracytoplasmic edema. HE × 300.
concentrations (15 to 30%) [5], and Fridovich [1] supposed that free radicals generated by HPO might cause lipid peroxidation in the plasma membrane, resulting in keratinocyte necrosis and subsequent epidermal thickening as a reparative change.

Apart from their severity, electron microscopic changes in the HPO-applied dorsal skin were common to HiR and WRs irrespective of the concentration of HPO. That is, necrotic keratinocytes were seen scattered in the spinous and basal layers, and necrotic keratinocytes were sometimes ingested by macrophages (Fig. 7). The basal layer contained a cluster of keratinocytes showing signs of shrinkage of the cell body and/or intracytoplasmic edema and was sometimes infiltrated by inflammatory cells, resulting in discontinuation of the basement membrane (Fig. 8).

In some portions, as seen light microscopically, there were spaces of various sizes filled with fluid between the epidermis and the dermis (Fig. 9). Marked degenerative changes were often detected in the capillary
endothelial cells in the superficial dermis (Fig. 10), although not clearly seen light microscopically. Such capillary endothelial damage may be due to toxic effects of HPO [5], and it is probable that the capillary endothelial damage may bring about edema, resulting in detachment of the epidermis from the dermis. This also seems to be related to the fact that keratinocytes in the basal layer were more frequently and highly damaged than those in other layers. Further investigations are now in progress to clarify the pathogenesis of HPO-induced dermatitis in HiRs after a single topical application.

In conclusion, WBN/Kob-Ht rats are more sensitive to HPO than Wistar rats and they seem to be useful for the investigation of the dermatotoxicity of environmental chemicals.

References

1. Fridovich, I. 1976. Oxigen radicals, hydrogen peroxide and