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Changes of superoxide dismutase and glutathione peroxidase in light damaged rat retina

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The changes in expression of copper-zinc superoxide dismutase (CuZn-SOD), manganese superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPX) in light-damaged rat retinas were examined. Sprague-Dawley rats (male, 6-weeks-old) were maintained on a cyclic photoperiod (12 hours light and 12 hours darkness) for 2 weeks. The illumination intensity during the light period was 80 lux. To induce light damage to the retina, a high-intensity illumination (3000-lux) was applied to the animals for 24 hours. After light exposure, the animals were returned to cyclic lighting. Eyes were enucleated 12 and 24 hours after light exposure started or 1, 3, and 7 days after light exposure ended. Eyes were fixed and embedded in paraffin wax. Tissues were cut into 4μm-thick sections. Sections were immunostained using antibody against CuZn-SOD, Mn-SOD, GPX and 8-hydroxy-deoxyguanocine (8-OHdG) as oxidative stress marker. 8-OHdG was observed in the outer nuclear layer (ONL) and retinal pigment epithelium (RPE) during light exposure. In light-damaged retinas CuZn-SOD labeling was up regulated in the ONL and RPE. Mn-SOD labeling was up regulated in rod inner segments (RIS) during light exposure and that in the RPE was up regulated after exposure. GPX labeling was observed in rod outer segments (ROS) during light exposure. GPX labeling was also observed in the RPE during and after light exposure. All three enzymes were observed in the outer retina, which suffered light damage, but occurred in defferent layers except within the RPE, in which case all three were expressed. These enzymes may play complementary roles as protective factors in light-damaged retinas.

key words: retina, copper-zinc superoxide dismutase, manganese superoxide dismutase, glutathione peroxidase, light damage

INTRODUCTION

It is known that intense light exposure can induce retinal damage and cause degeneration of rod outer segments (ROS) and the retinal pigment epithelium (RPE) [1]. Like many other organs, the retina itself has several lines of defense against light damage. There is increasing evidence that antioxidants play a major role in retinal protection against light exposure, although the mechanism of light induced damage in the retina is not well understood [2]. A variety of antioxidant enzymes and antioxidants exist in the retina [3-5], and it seems that each enzyme and antioxidant has a different role under conditions of oxidative stress induced by light exposure.

In this study, we immunohistochemically examined the precise localization of copper-zinc superoxide dismutase (CuZn-SOD), manganese superoxide dismutase (Mn-SOD), and glutathione peroxidase (GPX) within rat retinas under oxidative stress induced by light exposure, and compared these results with those obtained from normal rat retinas. The protective role of these three enzymes is also disucussed.

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MATERIALS AND METHODS

Model of light exposure.

The animals were exposed to high-intensity white light following a method described by Yamamoto and associates with minor modification [6]. Sprague-Dawley rats (male, 6-weeks-old) were obtained from Charles River Japan, Inc. (Kanagawa, Japan) and maintained on a cycle of 12 hours of light (80 lux) and 12 hours of darkness at 20°C for 2 weeks. The animals then were exposed to high-intensity illumination (3,000 lux) produced by white fluorescent bulbs for 24 hours at 25 ±1.5°C. After illumination, the

Figure 1. Localization of CuZn-SOD in normal and light-exposed rat retinas.
(a) Normal. (b) Light exposure for 12 hours. (c) Light exposure for 24 hours. (d) Post 1 day. (e) Post 3 days. (f) Post 7days after light exposure. Immunolabeling is up regulated in outer nuclear layer (arrows) and retinal pigment epithelium (arrowheads) by light exposure.

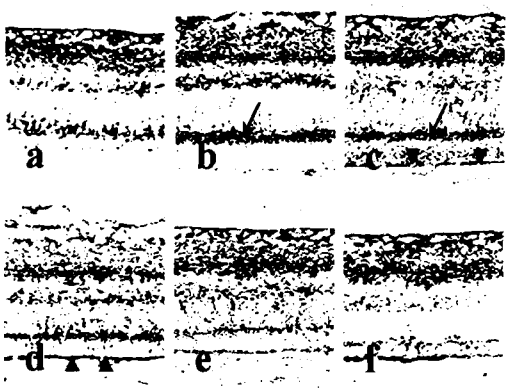

Figure 2. Localization of Mn-SOD in normal and light-exposed rat retinas.
(a) Normal. (b) Light exposure for 12 hours. (c) Light exposure for 24 hours. (d) Post 1 day. (e) Post 3 days. (f) Post 7days after light exposure. Immunolabeling is up regulated in rod inner segments (arrows) and retinal pigment epithelium (arrowheads) by light exposure.

animals were returned to a cyclic photoperiod at 20 °C during recovery.

The eyes were enucleated 12 and 24 hours after the start of light exposure or 1, 3, and 7 days after the completion of the light exposure. Rats raised under cyclic lighting without high-intensity light exposure served as controls.

Fixation of ocular

The rats were perfused through the left cardiac ventricle with 0.1 M phosphate buffer (pH 7.4), and then with 2% paraformaldehyde and 0.1% glutaraldehyde and 1% sucrose in the same buffer at 4° C. The eyes were removed and fixed with the same fixative for 6 hours at 4°C. All tissues were embedded in paraffin wax, and cut into 4-μm-thick sections parallel with the sagittal line.

Antibodies.

Dr Kohtaro Asayama (Department of Pediatrics, University of Occupation and Environmental Health) kindly gifted rabbit anti-rat CuZn-SOD, rat Mn-SOD and rat cellular GPX antibody [7, 8]. Mouse anti-8-hydroxy-deoxyguanosine (8OHdG) antibody was purchased from NOF-corporation (Tokyo, Japan).

Confirmation of oxidative stress

The degree of retinal damage was confirmed by monoclonal antibody against 8-OHdG, one of the major DNA base-modified products. Sections were stained with Avidin-biotinylated peroxidase complex (ABC) method using a kit (HISTOFINE, Nichirei, Tokyo, Japan).

Immunohistochemical analysis

The techniques for immunohistochemical staining have been described in detail elsewhere [6]. We used a Silver Enhancing kit (BBInternational Inc., Cardiff, UK) to detect enzymes. Briefly, deparaffinize sections were incubated overnight at 4°C with antibodies (1:1000) or with control antibody (non-immuno rabbit IgG). Sections were rinsed with KPBS, incubated with gold-conjugated secondary antibody (BBInternational Inc.) for 30 minutes, and then rinsed again. The antigen-antibody complexes were visualized by immersion into enhancing solution (BB International Inc.) for 5.

RESULTS

8-OHdG labeling

After light exposure for 12 and 24 hours, RPE cells, photoreceptor cells and ganglion cells were labeled with 8-OHdG, while cells labeled with 8-OHdG were not observed in the normal control retinas (data not shown). Thus we confirmed that the retinas suffered damage from oxidative stress induced by light exposure.

CuZnSOD labeling

CuZnSOD labeling was observed in the internal limiting membrane (ILM), ganglion cell layer (GCL), inner plexiform layer (IPL) and RPE in the normal control retinas (Fig. 1-a). After 12 and 24-hour exposure, the intensity of longitudinal labeling in the outer nuclear layer (ONL) and

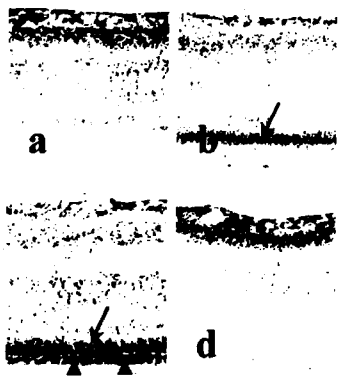

Figure 3. Localization of GPX in normal and light-exposed rat retinas. (a) Normal. (b) Light exposure for 12 hours. (c) Light exposure for 24 hours. (d) Post 1 day after light exposure. Immunolabeling is up regulated in rod outer segments (arrows) and retinal pigment epithelium (arrowheads) by light exposure.

RPE became stronger (Fig. 1-b, c). One day after light exposure, CuZnSOD labeling in the ONL became weaker (Fig. 1-d) and three days after light exposure, the labeling in the RPE also became weaker (Fig. 1-e). In retinas obtained 7 days after light exposure, the longitudinal labeling in the ONL became very weak or disappeared (Fig. 1-f).

Mn-SOD labeling

Mn-SOD labeling was observed in the ILM, GCL, IPL, OPL and inner nuclear layer (INL) (Fig.1-a) in the normal control retinas. After 12-hours exposure, labeling was seen in rod inner segments (RIS), and, after 24-hours exposure, labeling was seen in the RPE as well(Fig. 1-b, c). One day after light exposure, strong labeling was seen in the RPE; however, labeling in RIS became weak (Fig. 1-d). Labeling in the RPE was seen until 7day after light exposure, although the intensity of the labeling weakened (Fig. 1-e, f).

GPX labeling

GPX labeling was observed in the ILM, GCL, IPL and INL in normal contol retinas (Fig. 2-a). After 12 and 24-hours exposure, GPX labeling was clearly seen in the ROS and RPE (Fig. 2-b, c). One day after light exposure, the intensity of GPX labeling in ROS had diminished (Fig. 2-d).

DISCUSSION

The retina has several lines of defense against light damage and the various antioxidant enzymes existing in the retina have different roles under oxidative stress induce by light exposure [3-5]. In this study, we immunohistochemically examined the change in expression of CuZn-SOD, Mn-SOD and GPX, which are antioxidant enzymes, to determine the protective role of these enzymes in light-damaged rat retinas.

During and/or after intense light exposure, CuZn-SOD, MnSOD and GPX labeling were up-regulated in the outer retina; CuZn-SOD was up regulated in the ONL and RPE (Fig. 3); MnSOD, in the RIS and RPE (Fig. 4); and, GPX, in the ROS and RPE (Fig. 5). The outer retina suffered damage from oxidative stress induced by light exposure. CuZn-SOD, Mn-SOD and GPX might be up regulated to protect the outer segment and these enzymes may play an important role in retinal protection against oxidative stress. Furthermore, these enzymes up regulated by light exposure were observed in different layers from each other, although they were all expressed in RPE. Thus, CuZn-SOD, Mn-SOD and GPX may play complementary roles as protective factors in light-damaged retinas against light-induced oxidative stress.

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