




# Protective effect of Peroxiredoxin 6 (PRDX6) treatment on the rat model with corneal injury caused by ultraviolet ray

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## ABSTRACT

**Objective:** To study the protective effect of Peroxiredoxin 6 (PRDX6) treatment on the rat model with corneal injury caused by ultraviolet ray. **Methods:** SD male rats were selected as experimental animals and randomly divided into control group, model group and PRDX6 group, corneal injury models were established by UV irradiation, and they received PRDX6 intervention. The contents of oxidative stress molecules, apoptosis molecules and MMPs/TIMPs in corneal tissues were detected on the 12 d after intervention. **Results:** MDA, AOPP, Fas, FasL, Bax, Caspase-3, MMP2 and MMP9 contents in corneal tissue of model group were significantly higher than those of control group while T-AOC, SOD, GSH-Px, Bcl-2, Survivin, XIAP, TIMP1 and TIMP2 contents were significantly lower than those of control group; MDA, AOPP, Fas, FasL, Bax, Caspase-3, MMP2 and MMP9 contents in corneal tissue of PRDX6 group were significantly lower than those of model group while T-AOC, SOD, GSH-Px, Bcl-2, Survivin, XIAP, TIMP1 and TIMP2 contents were significantly higher than those of model group. **Conclusion:** PRDX6 has inhibitory effect on the oxidative stress and apoptosis in corneal injury process caused by ultraviolet ray.

## 1. Introduction

Corneal injury caused by ultraviolet ray is a common type of eye injury, long-term eye exposure to ultraviolet light can cause corneal damage, it is characterized by foreign body sensation and pricking as well as photophobia and lacrimation, and severe cases can cause corneal edema, opacity and detachment, and affect vision level[1,2]. The oxidative stress injury is the important mechanism for ultraviolet to cause corneal damage, and continuous ultraviolet action will increase the formation of oxygen free radicals in local cornea tissue, which have oxidizing reaction with lipid and protein in cornea, and then cause tissue damage[3,4]. Therefore, anti-oxidation is an important therapy for corneal ultraviolet damage. Peroxiredoxin-6 (PRDX6) is a peroxidase family member with glutathione peroxidase activity, which can scavenge oxygen free radicals and reduce oxidative stress damage[5]. In the following studies, we analyzed the protective effects of PRDX6 treatment on the rat model with corneal injury caused by ultraviolet ray.

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## 2. Experimental materials and methods

### 2.1 Experimental materials

A total of 24 SD male rats were selected as experimental animals and provided by experimental animal center of Southwest Medical University, license key was SCXK (Sichuan) 2013-17, animal experiments passed the hospital ethical review, and animal experiment operation and animal treatment after death were carried out according to rules. Compound Tropicamide Eye Drops were bought from Shenyang Sinqi Pharmaceutical Company, PRDX6 protein was bought from Sigma Company, and enzyme-linked immunosorbent assay kit and radioimmunoprecipitation kit were purchased from Nanjing Jiancheng Bioengineering Institute.

### 2.2 Experimental methods

#### 2.2.1 Experimental grouping and intervention

SD rats were randomly divided into control group, model group and PRDX6 group, and the model group and PRDX6 group were established into cornea uv damage models according to the

following methods: intraperitoneal injection of 40 mg/kg nembatal, dropping Compound Tropicamide Eye Drops in the right eye after anesthesia, fixing the right eye with tape to make it in open state, and irradiating it with UV lamp for 20 min every time and four days in a row. The model group received aseptic PBS buffer for right eye drop, and PRDX6 group received PRDX6 solution for right eye drop, four times a day for 12 consecutive days.

### 2.2.2 Protein content detection

Rats were executed and anatomized to get the cornea tissue and add it in RIPA lysate to extract protein, radioimmunoprecipitation kit was used to detect the levels of MDA, AOPP, T-AOC, SOD and GSH-Px, and enzyme-linked immunosorbent assay kit was used to detect the levels of Fas, FasL, Bax, Caspase-3, Bcl-2, Survivin, XIAP, MMP2, MMP9, TIMP1 and TIMP2.

### 2.3 Statistical methods

SPSS 20.0 software was used for data input and variance analysis, and  $P < 0.05$  indicated statistical significance in differences in analysis results.

## 3. Results

### 3.1. Oxidative stress molecule contents in corneal tissue

Analysis of oxidative stress molecules MDA ( $\mu\text{mol/L}$ ), AOPP ( $\mu\text{mol/L}$ ), T-AOC ( $\mu\text{mol/L}$ ), SOD (U/mL) and GSH-Px (U/mL) in corneal tissue among three groups of rats was as follows: MDA and AOPP contents in corneal tissue of model group were significantly higher than those of control group while T-AOC, SOD and GSH-Px contents were significantly lower than those of control group; MDA and AOPP contents in corneal tissue of PRDX6 group were

significantly lower than those of model group while T-AOC, SOD and GSH-Px contents were significantly higher than those of model group. Differences in MDA, AOPP, T-AOC, SOD and GSH-Px contents in corneal tissue were statistically significant among three groups of rats ( $P < 0.05$ ).

### 3.2 Apoptosis molecule expression in corneal tissue

Analysis of apoptosis molecules Fas (ng/mL), FasL (ng/mL), Bax (pg/mL), Caspase-3 (ng/mL), Bcl-2 (pg/mL), Survivin (ng/mL) and XIAP (ng/mL) in corneal tissue among three groups of rats was as follows: Fas, FasL, Bax and Caspase-3 contents in corneal tissue of model group were significantly higher than those of control group while Bcl-2, Survivin and XIAP contents were significantly lower than those of control group; Fas, FasL, Bax and Caspase-3 contents in corneal tissue of PRDX6 group were significantly lower than those of model group while Bcl-2, Survivin and XIAP contents were significantly higher than those of model group. Differences in Fas, FasL, Bax, Caspase-3, Bcl-2, Survivin and XIAP contents in corneal tissue were statistically significant among three groups of rats ( $P < 0.05$ ).

### 3.3 MMPs/TIMPs expression in corneal tissue

Analysis of MMPs/TIMPs MMP2, MMP9, TIMP1 and TIMP2 in corneal tissue among three groups of rats was as follows: MMP2 and MMP9 contents in corneal tissue of model group were significantly higher than those of control group while TIMP1 and TIMP2 contents were significantly lower than those of control group; MMP2 and MMP9 contents in corneal tissue of PRDX6 group were significantly lower than those of model group while TIMP1 and TIMP2 contents were significantly higher than those of model group. Differences in MMP2, MMP9, TIMP1 and TIMP2 contents in corneal tissue were statistically significant among three groups of rats ( $P < 0.05$ ).

Table 1.

Comparison of oxidative stress molecules in corneal tissue among three groups of rats.

Groups	n	MDA	AOPP	T-AOC	SOD	GSH-Px
Control group	8	1.05±0.15	0.77±0.09	6.79±0.93	27.82±4.42	22.31±3.49
Model group	8	2.62±0.36 <sup>*</sup>	2.26±0.32 <sup>*</sup>	2.21±0.34 <sup>*</sup>	9.24±1.02 <sup>*</sup>	7.69±0.89 <sup>*</sup>
PRDX6 group	8	1.67±0.20 <sup>#</sup>	1.33±0.17 <sup>#</sup>	4.48±0.62 <sup>#</sup>	19.69±2.31 <sup>#</sup>	17.64±2.03 <sup>#</sup>

<sup>\*</sup>: model group vs. control group, differences in indexes were statistically significant; <sup>#</sup>: PRDX6 group vs. model group, differences in indexes were statistically significant;

Table 2.

Pro-apoptosis molecule contents in corneal tissue of three groups of rats.

Groups	n	Fas	FasL	Bax	Caspase-3
Control group	8	1.38±0.18 <sup>*</sup>	0.92±0.11 <sup>*</sup>	96.72±10.25 <sup>*</sup>	1.77±0.20 <sup>*</sup>
Model group	8	4.59±0.67 <sup>#</sup>	3.09±0.52 <sup>#</sup>	332.95±52.93 <sup>#</sup>	5.40±0.78 <sup>#</sup>
PRDX6 group	8	2.03±0.28	1.55±0.18	167.35±20.35	2.52±0.35

<sup>\*</sup>: model group vs. control group, differences in indexes were statistically significant; <sup>#</sup>: PRDX6 group vs. model group, differences in indexes were statistically significant.

Table 3.

Anti-apoptosis molecule contents in corneal tissue of three groups of rats.

Groups	n	Bcl-2	Survivin	XIAP
Control group	8	673.21±89.35 <sup>*</sup>	2.94±0.35 <sup>*</sup>	1.92±0.31 <sup>*</sup>
Model group	8	245.63±31.47 <sup>#</sup>	1.02±0.15 <sup>#</sup>	0.68±0.09 <sup>#</sup>
PRDX6 group	8	498.62±62.38	2.26±0.34	1.44±0.18

<sup>\*</sup>: model group vs. control group, differences in indexes were statistically significant; <sup>#</sup>: PRDX6 group vs. model group, differences in indexes were statistically significant;

Table 4.

MMPs/TIMPs contents in corneal tissue of three groups of rats.

Groups	n	MMP2	MMP9	TIMP1	TIMP2
Control group	8	97.41±11.32	127.62±14.58	75.51±9.34	60.21±8.34
Model group	8	304.54±46.24 <sup>*</sup>	479.31±57.67 <sup>*</sup>	33.56±6.49 <sup>*</sup>	26.58±4.26 <sup>*</sup>
PRDX6 group	8	152.33±17.58 <sup>#</sup>	203.44±28.39 <sup>#</sup>	59.23±6.95 <sup>#</sup>	45.52±5.92 <sup>#</sup>

<sup>\*</sup>: model group vs. control group, differences in indexes were statistically significant; <sup>#</sup>: PRDX6 group vs. model group, differences in indexes were statistically significant.

#### 4. Discussion

Ultraviolet radiation is a common cause of corneal injury, and the continuous exposure of the cornea to ultraviolet radiation can result in an increase of oxygen free radicals and excessive activation of oxidative stress. The oxygen free radicals generated in the local cornea can cause lipid and protein oxidation in the cells, which will generate products MDA and AOPP, and cause the damage to cell structure and function[6,7]. In the study, analysis of the oxidative stress product contents in corneal tissue of model group showed that the contents of MDA and AOPP in the corneal tissues of the model group were significantly higher than those of the control group. This indicates that oxidative stress response activation is an important pathological link in the corneal injury caused by ultraviolet radiation, and the generation of oxygen free radicals and corresponding oxidation products increase in the process of corneal injury. PRDX family is a class of non-selenium-dependent peroxidase with strong antioxidant activity, PRDX6 expression is relatively abundant in epithelium, epidermis and cornea, and it can mitigate the oxidative stress reaction in local tissue[8,9]. Further analysis of the effects of PRDX6 on oxidative stress response in corneal tissue showed that the contents of MDA and AOPP in the corneal tissues of PRDX6 group were significantly lower than those in the model group. This indicates that PRDX6 has inhibitory effect on the generation of oxygen free radicals and the activation of oxidative stress response in the process of corneal ultraviolet damage.

In the process of corneal damage caused by ultraviolet ray, the large amount of oxygen free radicals will not only cause oxidative damage to the tissues, but also cause a lot of antioxidant enzymes to be consumed. SOD and GSP-Px are important antioxidant enzymes in the body, which can ensure the antioxidant capacity of local tissues[10,11]. In the study, analysis the effect of uv irradiation on antioxidant capacity and antioxidant enzyme contents in corneal tissue showed that the contents of T-AOC, SOD and GSH-Px in

the corneal tissue of the model group were significantly lower than those of the control group. This shows that in the process of corneal damage caused by ultraviolet radiation, various antioxidant enzymes are consumed and the antioxidant capacity significantly reduces. PRDX6 is a bifunctional protein in the PRDX family, which has the catalytic activity of both GSH peroxidase and phospholipase A2, and is able to take glutathione as electron donor to degrade hydrogen peroxide, then improve the antioxidant capacity of local tissue, and reduce oxidative stress damage to the tissue[12,13]. After PRDX6 treatment, it was found that the contents of T-AOC, SOD and GSH-Px in the corneal tissues of PRDX6 group were significantly higher than those of the model group. This shows that PRDX6 can reduce the antioxidant enzyme consumption and enhance the antioxidant capacity in the process of corneal ultraviolet damage.

The large amount of oxygen free radicals in corneal tissues will not only directly cause oxidative damage to cells, but also activate cell apoptosis and affect cell function. Mitochondrial pathway and death receptor pathway are the important mechanisms to regulate apoptosis, the former is regulated by Bax/Bcl-2, the latter is regulated by Fas/FasL, and both ways can activate Caspase-3 and mediate apoptosis through the downstream cascade activation reaction[14,15]. Survivin and XIAP are important anti-apoptosis molecules in cells, which can antagonize the catalytic activity of many kinds of Caspase molecules and inhibit apoptosis[16,17]. In the study, the analysis of apoptosis in the process of corneal injury caused by ultraviolet showed that Fas, FasL, Bax and Caspase-3 contents in corneal tissue of model group were significantly higher than those of control group while Bcl-2, Survivin and XIAP contents were significantly lower than those of control group. This indicates that the uv irradiation can activate the apoptosis process in corneal tissue and thereby cause corneal damage. Further analysis of the effect of PRDX6 intervention on the apoptosis in the process of corneal injury caused by ultraviolet showed that Fas, FasL, Bax and Caspase-3 contents in corneal tissue of PRDX6 group were significantly lower than those of model group while Bcl-2, Survivin and XIAP contents were significantly higher than those of model group. This indicates that

PRDX6 can inhibit the apoptosis of mitochondrial pathway and death receptor pathway in the process of corneal ultraviolet damage. There is tissue reconstruction and repair after corneal ultraviolet irradiation damage, and in this process, the abnormal degradation and deposition of extracellular matrix can cause scar healing[18]. MMPs and their inhibitory molecules TIMPs are important molecules for regulating the synthesis and degradation of extracellular matrix. MMP2 and MMP9 can degrade gelatin, collagen, elastin and laminin in extracellular matrix and promote scar formation; TIMP1 and TIMP2 can be combined with MMP2 and MMP9 to form covalent bonding and inhibit their hydrolysis activity, which has inhibitory effect on scar formation[19,20]. In the study, the analysis of MMPs/TIMPs expression in the process of corneal injury caused by ultraviolet showed that MMP2 and MMP9 contents in corneal tissue of model group were significantly higher than those of control group while TIMP1 and TIMP2 contents were significantly lower than those of control group. This indicates that the uv irradiation can activate the apoptosis process in corneal tissue and thereby cause corneal damage. Further analysis of the effects of PRDX6 on MMPs/TIMPs expression showed that MMP2 and MMP9 contents in corneal tissue of PRDX6 group were significantly lower than those of model group while TIMP1 and TIMP2 contents were significantly higher than those of model group. This indicates that PRDX6 can regulate the expression of MMPs/TIMPs in the process of corneal ultraviolet damage, and thereby inhibit scar healing.

The activation of oxidative stress and apoptosis is closely related to the corneal injury induced by ultraviolet ray; PRDX6 can inhibit oxidative stress response and apoptosis in the process of corneal damage induced by ultraviolet light, and can also regulate MMPs/TIMPs expression and inhibit scar healing.

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