Biomedicine & Pharmacotherapy 96 (2017) 524–534

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

A mouse dry eye model induced by topical administration of the air pollutant particulate matter 10

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ARTICLE INFO

Keywords:
PM_{10}
Ocular surface
Tear film
Dry eye

ABSTRACT

Aim: To introduce a novel dry eye mouse model induced by topical administration of the air pollutant particulate matter 10 (PM_{10}).

Method: A total of 60 male BALB/c mice were used in this study and divided into two groups: group A (PBS eye drops, n = 30) and group B (PM_{10} eye drop group, n = 30). Each treatment was dosed four times a day, every time 50ul with the concentration of 5 mg/ml PM10, for 14 consecutive days in the right eye. The clinical manifestations of dry eye were measured before therapy and 4, 7 and 14 days post-treatment respectively, which included the tear volume, tear break-up (BUT) time, corneal fluorescein staining, rose bengal staining, Lissamine Green staining and inflammatory index. Eye samples were collected on D14 and examined by histologic light microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM), corneal cytokeration 10 (K10) immunnostaining, and tumor necrosis factor-α (TNF-α), NF-κB-p65 and NF-κB Western Blot analysis.

Results: At 0d, 7d and 14d, there were no statistical changes in tear volume, BUT after treatment ($P > 0.05$) with PBS in group A. In group B, all items showed statistical differences at each time point ($P < 0.05$). At 14d after therapy, the fluorescein staining score of group B was higher than group A ($P < 0.05$). The score of rose bengal staining and Lissamine Green staining in group B was also higher than that in group A ($P < 0.05$). The number of mean layers of corneal epithelial cells in the group A was significantly lower than that in the group B ($P < 0.05$). TEM and SEM revealed that the number of corneal epithelial microvilli were drastically reduced in group B. The number of corneal chondriosome/desmosomes was also reduced in group B by TEM. PM_{10} induced apoptosis in the superficial and basal corneal epithelium, and leaded to abnormal differentiation and proliferation of the ocular surface with higher expression levels of K10 and reduced number of goblet cells in the conjunctival fornix in group B. PM_{10} significantly increased the levels of TNF-α, NF-κB-p65 and NF-κB in the cornea.

Conclusion: PM_{10} can damage the tear film function and cause the destruction of the structural organization of ocular surface in mice. Topical administration of PM_{10} in mice induces ocular surface changes that are similar to those of dry eye in humans, representing a novel model of DES.

1. Introduction

Air pollution is becoming a serious global issue with the continuous development of industrialization. A growing number of studies have shown that air pollution can harm people's health. Atmospheric particulate matter (particular matter PM), one of the major components of air pollution, is a mixture of liquid and solid materials of different sizes and chemical characteristics, including dust, dirt, smoke and droplet. In

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http://dx.doi.org/10.1016/j.biopha.2017.10.032
Received 13 August 2017; Received in revised form 3 October 2017; Accepted 9 October 2017
0753-3322/ © 2017 Published by Elsevier Masson SAS.

2013, WHO (Health Organization World) reported that the impact of atmospheric particulate matter on the human body is mainly from PM_{10} and $PM_{2.5}$. Particles with diameter less than 10 μm can reach the bronchi and alveoli in the lung. Particulate matters with diameter less than 2.5 have stronger penetration ability, and may reach the bronchial capillary wall and interfere with gas exchange in the lung [1]. Atmospheric particulate matter can also have a lot of effects on human health, including increasing rate of mortality, asthma, COPD, atherosclerosis and diabetes [2–4].

Dry eye syndrome is a tear and ocular surface disease caused by a variety of factors, including ocular surface discomfort symptoms, changes in visual acuity and instability of tear film, and is often accompanied by potential ocular surface damage, tear osmotic pressure elevation and ocular inflammation [5]. Common symptoms include eye dryness, fatigue, itching, foreign object sensation, burning pain, sticky secretions, ancraophobia, photophobia and increased sensitivities to external stimuli. Sometimes because of lack of basic tear, excessive eye dryness stimulates the reflex lacrimal secretion, causing frequent tearing, even eye redness, hyperemia, and keratinization. With the further development of corneal epithelial exfoliation and filament attachment, eye dryness can cause conjunctival lesions, and affect vision [6].

An increasing number of epidemiological studies have indicated that air pollution can cause eye discomfort. Chang et al. found that the increase in PM_{10} would lead to an increased rate of outpatient visits [7]. And another large sample (about 500 people) epidemiological survey showed that the Schirmer I test and BUT time were lower than normal values of the population in the area of serious air pollution, among which 50% reported eye irritation and other symptoms. The study also found that ocular surface subclinical symptoms were increased in the tourists of air pollution areas, such as burning, eye fatigue, foreign object sensation, eye itching and other symptoms [8]. Some small sample epidemiological survey also showed that some of the ocular surface symptoms were related to air pollution [9–12]. So, whether particle matter can lead to dry eye becomes a serious issue. This study, for the first time, explored the effects of particulate matter 10 (PM_{10}) eye drops on ocular surface structure and tear function in mice, and established an animal model and a scientific basis for clinical prevention and treatment.

2. Materials and methods

2.1. Human corneal epithelial cell culture and assays

The simian virus-40 (SV40) transformed human cornea epithelial (HCE) cell line was obtained from RIKEN Biosource Center (Tokyo, Japan). The culture medium was Dulbecco's modified Eagle's medium (DMEM) (F-12; Invitrogen) with 6% fetal bovine serum, plus 7.5 mg/ml insulin and 10 ng/ml epidermal growth factors.

2.2. Cell counting kit-8 (CCK-8) assay

The cell proliferation was assayed with the cell counting kit-8 (CCK-8) assay (Tokyo, Japan). 5000 cells/well HCE cells (HCECs) in 96-well plates were treated with 100 μg/ml to 10 mg/ml PM_{10} (100 μ100 PM to 10ates were trea, 1.0 mg/ml, 2.0 mg/ml, 5.0 mg/ml or 10.0 mg/ml) for 24 h, and culture medium as control [13]. 10 Pl CCK-8 solution (Dojindo Co., Tokyo, Japan) was added to every well, and the absorbance at 450 nm was measured after 24 h incubation, as previously described [14].

2.3. Wound closure assay for assessing migration

Migration of HCECs was measured by a wound-healing assay as reported [15]. Briefly, a 0.6 mm-width uniform scratch wound was created in a confluent HCEC monolayer by a pipette tip. The cells were further incubated in the presence of 1% BSA or 5.0 mg/ml PM_{10} for 0, 8, and 12 h, and photographed. Wound closure was used to determine cell migration.

2.4. Animal preparation

Totally 60 male specific pathogen free (SPF) BALB/c mice (18–20 g, purchased from Laboratory Animal Center of Xi'an Jiao Tong University College of Medicine, Xi'an, China) were used for this study. No abnormality was found in the anterior segment and fundus when examined with slit lamp microscope and fundus examination. Mice were housed in standard environment throughout the study [16]. Throughout the study, the animals were kept in a stable environment: room temperature 25 °C ± 1 °C, relative humidity 60% + 10%, adequate food and water. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by the animal ethics committee of Xi'an Jiao Tong University College of Medicine (Xi'an, China).

2.5. Acquisition of PM10 and preparation of eye drops

PM_{10} were provided by Xi'an environmental monitoring station. During October 1 st to October 31 st 2015, a super station in Xi'an City acquired PM samples with sizes of 10 μm using TH-16A four channel atmospheric particulate automatic sampler (Wuhan Tianhong instrument Ltd), and filtered through Whatman PTFE membrane. Sampling period was 22 h a day from 10:30 am to 8:30 am next day continuously. PTFE membranes containing PM_{10} were cut into 1cm × 1 cm pieces, immersed in distilled water and ultrasonic oscillated for 45 min × 3 times. After 6 layers of gauze filtration, samples were vacuum frozen dried and weighted [17]. Samples were then stored at 4 °. Preparation of PM eye drops: PM_{10} were diluted in sterile PBS to the concentration of 5 mg/ml, and vortex with ultrasound. Preservative benzyl bromide was added to two groups of eye drops (PM and PBS) with concentration controlled at 0.005%. Eye drops were kept at 4 °.

2.6. Animal experimental procedure

60 mice were divided into two groups: Group A, PBS treatment, n = 30, right eyes, 4 times daily and Group B, 5.0 mg/ml PM_{10} treatment, n = 30, right eyes, 4 times daily. The frequency and doses of PM treatment were determined previously with a pilot experiment. Before the treatment, all mice were confirmed to be free of any ocular diseases. Body weight, eyeball weight and extra-orbital lacrimal gland weight, phenol red thread test, fluorescein staining, tear film break-up time (BUT) test, rose bengal staining, inflammatory index and HE staining were performed sequentially before and 4, 7, and 14 days after the treatment. All mice were euthanized on day 14, and the eyes were harvested for histological analysis and western blot.

2.7. Tear volume, fluorescein and BUT measurement

Phenol red thread tear test with phenol red-impregnated cotton threads (FCI Ophthalmics, Pembrooke, MA, USA) was used to measure tear amount on days 0, 4, 7, and 14 post-treatment. Tear volume, Fluorescein and BUT were measured as previously described [18,19]. Measurements of basal tear secretion in rats were performed at the same time point (9pm) for each group of mice. With the head fixed, the eyelid was pulled down lightly to expose the conjunctival sac. The phenol red thread was bend 5 mm to the end, and placed under the 1/3 medial palpebral conjunctival sac. 15 s later, the thread was withdrawn, and tear soaked red phenol red thread length was measured. For each eye, the measurements were repeated 3 times and averaged. After the completion of the test, the eyes were closed to avoid excessive exposure and ocular surface irritation. Fluorescent staining: 5ul 0.1% fluorescein sodium fluorescein staining solution was dropped onto rat

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