Received: 30.12.2008 **Accepted: 9.6.2009**

Original Article

Prophylactic ophthalmic bethametazone for sulfur mustard-induced ocular injury

Mostafa Naderi[a], Gholam-Reza Kaka[b,c], Khosro Jadidi[a], Hamid-Reza Khoddami-Vishteh[d], Navvab Shamspour[d], Seyed-Homayoun Sadraie[c]*

Abstract

BACKGROUND: The present study sought to evaluate the prophylactic effect of bethametazone on sulfur mustard (SM)-induced ocular morphometric damage in the rabbit eye.

METHODS: Twenty five healthy New Zealand white rabbits were divided into 4 groups of normal (not exposed to SM or solution), solution (exposed to solution), SM (exposed to SM), and prophylactic bethametazone (received eye solution of bethametazone then exposed to SM solution; then treated for 2 weeks)**.** On the day 14 after exposure, five-micron sections were stained with haematoxylin and eosin for light microscopy evaluation. The ocular morphometric characteristics in the study groups were compared to determine the prophylactic effects of the bethametazone.

RESULTS: Bethamethazone could protect eyes from SM effect by means of decrease in changes in number of Keratocyte in 10000 μm^2, thickness of cornea (µm), thickness of corneal epithelium (µm), number of meibomian gland's cells in 2500 μm^2, thickness of palpebral conjuctival epithelium (µm), thickness of epithelial of palpebral skin (µm), number of epithelial layers of palpebral skin, and number of goblet cells in conjunctival sac in 1000 µm.

CONCLUSIONS: Bethametazone may have a prophylactic effect on the early lesions of the eye of the rabbit due to SM exposure.

KEYWORDS: Betamethasone, Sulfur Mustard, Ocular Lesions.

JRMS 2009; 14(5): 291-295

The skin, eye, and respiratory system are three major targets for the local toxic effects of sulfur mustard (SM).[1] Out of the three, the eye is the most sensitive organ to SM,[1] and this marked susceptibility is attributable to several ocular features, including the aqueous-mucous surface of the cornea and conjunctiva as well as the high turnover rate and intense metabolic activity of the corneal epithelial cells.[2] Ocular injuries appear in 75-90% of all mustard gas casualties, with reports of delayed ocular morbidity appearing years later.[3] The pathologic mechanisms underlying SM-induced tissue injury remain undefined[4]; nonetheless, previous studies have demonstrated that SM may activate protease and cyclooxygenase, which can produce free radicals.[5] These harmful species are known to cause oxidative damage to a number of molecules in cells, including membrane lipids, proteins, and nucleic acids.[6]

One study to assess the SM effects on rabbit eyes, showed that SM exposure initiates typical clinical symptoms within 2-6 hours, characterized by eye closure, eyelid swelling, conjunctival hyperemia, corneal erosions and inflamma-

[a] Department of Ophthalmology, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[b] Research Center for Chemical Injuries, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[c] Department of Anatomy, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[d] Medicine and Health Promotion Institute, Tehran, Iran.
* Corresponding Author
E-mail: gh_kaka@yahoo.com

tion. The clinical signs were significantly dose-dependent and reached a peak at 24-72 hours post exposure. Biochemical evaluation of the aqueous humor exhibited an inflammatory reaction and oxidative stress at 4 hours after exposure, subsiding at 28 hours after exposure. Histological examination of corneas at 48 hours revealed epithelial denudation and marked stromal edema, accompanied by cellular infiltration. Epithelial regeneration started after 72 hours, and recovery was almost completed within 1-2 weeks, depending on the HD dose. A second phase of pathological processes started as early as two weeks post exposure and was characterized by corneal edema, opacity, recurrent erosions and neovascularization. The delayed injuries were found in 25% and 40% of the eyes respectively, and when appearing, were more severe than the initial ones.[7] One of the most prominent histological features in the early phase of SM exposure is loss of polarity of corneal epithelial basal cells,[8] following by recurrent oedema of the cornea as more delayed response.[9] SM causes chronic and delayed destructive lesions in the cornea.

Methods

Fifteen healthy New Zealand white rabbits of both sexes were used in this experimental study. The animals were approximately 4 months old with an initial body weight range of 1.8 to 2.2 kg. The study protocol was approved by the Committee for Animal Care of Baqiyatallah University of Medical Sciences. The animals were maintained in dust free bedding cages in a controlled environment with a specified range of temperature (65°F-65°F) and relative humidity (30% or more).

Twenty five healthy New Zealand white rabbits were divided into 4 groups of normal (not exposed to SM or solution), solution (exposed to solution), SM (exposed to SM), and prophylactic bethametazone (received eye solution of bethametazone then exposed to SM solution; then treated for 2 weeks).

On day 14, the animals were sacrificed by pentobarbital overdose via ear vein injections. The eyes were enucleated and were subsequently either fixed in a 4% neutral buffered paraformaldehyde solution or frozen for further analysis. Following fixation, the eyes were processed for paraffin embedding. Five-micron-thick sections were stained with haematoxylin and eosin (H&E) for light microscopy evaluation.

An experienced pathologist evaluated the thickness of the cornea, epithelium cornea, endothelium, limbos epithelium, and epithelium conjunctive in conjunction with the layer frequencies of the epithelium cornea, limbos epithelium, and epithelium conjunctive as well as the frequency of meibomian gland cells using a Motic light microscope.

Statistical analyses were done using SPSS software (Statistical Package for Social Sciences, version 13.0, SPSS Inc., Chicago, Ill, USA). The data were expressed as mean ± standard deviation or percentage where appropriate. ANOVA was used to compare ocular morphometric characteristics of the study groups. A p-value of less than 0.05 was considered significant.

Results

Effect of SM Exposure

The SM exposure group was significantly different from normal and solution groups by means of number of Keratocyte in 10000 μm^2, thickness of cornea (µm), thickness of corneal epithelium (µm), and number of corneal epithelial layers (Table 1). It was also significantly different from normal and solution groups by means of thickness of limbal epithelium (µm) and thickness of descemet's memberain and endothelium (µm) (Table 1). Moreover, the SM exposure group was significantly different from normal and solution groups by means of number of meibomian gland's cells in 2500 μm^2, thickness of palpebral conjuctival epithelium (µm), number of palpebral conjuctival epithelial layers, thickness of epithelial of palpebral skin (µm), number of epithelial layers of palpebral skin, number of goblet cells in conjunctival sac in 1000 µm (Table 1).

Table 1. Comparisons of mean ± SEM ocular morphometric characteristics between study groups

	Normal	Solution	SM	Bethametazone
Number of Keratocyte in 10000 µm^2	10.01 ± 0.52	10.50 ± 0.50	3.00 ± 1.00 ***	7.62 ± 1.40 †
Thickness of cornea (µm)	419.53 ± 27.68	417.23 ± 38.89	677.01 ± 63.60**	458.25 ± 16.27 ††
Thickness of corneal epithelium (µm)	28.19 ± 1.26	24.94 ± 1.49	11.41 ± 1.93***	26.96 ± 3.37 ††
Number of corneal Epithelial Layers	3.58 ± 0.15	3.22 ± 0.40	1.67 ± 0.33 ***	2.83 ± 0.44
Thickness of limbal epithelium (µm)	4.67 ± 3.51	32.00 ± 3.00	16.00 ± 2.00*	30.25 ± 5.26
Number of limbal Epithelial layers	14.72 ± 0.42	4.50 ± 0.50	3.00 ± 0.57	4.25 ± 0.47
Thickness of bulbar conjunctival Epithelium (µm)	2.60 ± 1.65	14.00 ± 1.00	18.00 ± 2.00	14.00 ± 2.08
Number of bulbar conjunctival epithelial layers	9.14 ± 0.24	2.50 ± 0.50	3.50 ± 0.50	2.67 ± 0.33
thickness of descemet's memberain and endothelium (µm)	34.67 ± 0.78	9.33 ± 0.88	6.40 ± 0.77*	7.24 ± 0.95
Number of meibomian gland's cells in 2500 µm^2	11.50 ± 0.64	11.50 ± 0.64	7.75 ± 0.47**	12.83 ± 0.30 †††
Thickness of palpebral conjuctival epithelium (µm)	21.00 ± 1.08	22.00 ± 1.22	28.71 ± 1.18***	19.00 ± 4.35 †
Number of palpebral conjuctival epithelial layers	3.75 ± 0.25	3.40 ± 0.24	5.00 ± 0.30*	4.00 ± 0.44
Thickness of epithelial of palpebral Skin (µm)	28.25 ± 0.94	29.20 ± 2.03	51.00 ± 2.43***	30.00 ± 13.05 †
Number of epithelial layers of palpebral Skin	4.25 ± 0.25	3.40 ± 0.92	7.42 ± 0.61**	4.00 ± 1.52†
Number of goblet cells in conjunctival sac in 1000 µm	46.50 ± 1.84	46.40 ± 1.86	7.28 ± 0.86***	25.16 ± 0.83†††

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$ for comparison between SM and normal and solution groups
† $p < 0.05$
†† $p < 0.01$
††† $p < 0.001$ for comparison between bethametazone and SM groups

Prophylactic Effect of Bethamethazone

Bethamethazone group was significantly different from SM exposure group by means of number of Keratocyte in 10000 µm^2, thickness of cornea (µm), and thickness of corneal epithelium (µm) (Table 1). Bethamethazone group was also significantly different from SM exposure group by means of number of meibomian gland's cells in 2500 µm^2, thickness of palpebral conjuctival epithelium (µm), thickness of epithelial of palpebral skin (µm), number of epithelial layers of palpebral skin, and number of Goblet cells in conjunctival sac in 1000 µm (Table 1).

Discussion

According to the results of this study, bethamethazone can protect eyes from SM effect by decreasing changes in number of Keratocyte in 10000 µm^2, thickness of cornea (µm), thickness of corneal epithelium (µm), number of meibomian gland's cells in 2500 µm^2, thickness of palpebral conjuctival epithelium (µm), thickness of epithelial of palpebral skin (µm), number of epithelial layers of palpebral skin, and number of goblet cells in conjunctival sac in 1000 µm.

In line with the present study, previous studies have shown that steroids may be potential candidates for the pretreatment of ocular lesions following SM exposure.[10] Betamethasone is known to modulate the development and propagation of inflammation; it not only decreases the production of inflammatory mediators (cytokines and chemokines) but also increases the synthesis of lipocortin and inhibits the production and decreases the induction of enzymes producing inflammatory mediators.[11] Betamethasone, therefore, attenuates both the initiation and the propagation of

inflammation. With respect to SM exposure, steroids have been previously recommended for the treatment of SM lesions only after re-generabbition of the epithelium, or after the appearance of anterior chamber uveitis.[11]

It must be noted, however, that the mechanism of SM-induced injuries has hitherto eluded the scientific community and no effective drug is known against the local and systemic lesions begotten by SM.[4] The best protection remains contact avoidance and in the event of contact, rapid decontamination or detoxification of the contaminated site.[1-4]

Although the pharmacological response to topical steroids in rabbits may be different from that in humans, we believe that our findings are significant in emergency conditions, for example if people should enter area of known SM contamination.

The present study carried out in vivo and to our knowledge there is no previous in vivo published document for the prophylactic use of betamethasone in SM-induced lesions.

It should be noted that the mechanisms of the prophylactic effects of bethametazone in SM-induced eye lesions were not evaluated in this study. Another limitation for this study was the use of a liquid solution of SM, not an aerosol exposure. Although the primary route of corneal exposure would be to aerosolized SM, and not to direct instillation of a SM solution into the eye, it was prohibitively expensive for our research center to perform controlled aerosolized exposures, and this study should be considered as a pilot-study.

Conclusions

Given that humans cannot be tested in future research and on the basis of the findings of the present study, betamethasone can be used as an effective prophylaxis against the hazardous effects of SM on the eye if the risk in an emergency can be justified.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

GRK, MN, and SHS carried out the design and coordinated the study. MN and KJ participated in most of the experiments. HRKV and NS prepared the manuscript. SHS provided assistance in the design of the study and participated in the revision of the manuscript. HRKV and NS provided assistance in data analysis. All authors have read and approved the content of the manuscript.

References

1. Geeraets WJ, Abedi S, Blanke RV. Acute corneal injury by mustard gas. South Med J 1977;70(3):348-50.
2. Balali M. Clinical and laboratory findings in Iranian fighters with chemical gas poisoning. In: Heyndrickx A, editor. Proceedings of the First World Congress on New Compounds in Biological and Chemical Warfare. Ghent: Rijksuniversiteit; 1984. p. 254-9.
3. Balali-Mood M, Navaeian A. Clinical and paraclinical findings in 233 patients with sulfur mustard poisoning. In: Heyndricks B, editor. Proceedings of the Second World Congress on New Compounds in Biological and Chemical Warfare. Ghent: Rijksuniversiteit; 1986. p. 464-73.
4. Smith WJ, Gross CL. Early events in the pathology of sulfur mustard in vitro: approaches to intervention. Proceedings of the NATO, Panel VIII Meeting, Grenoble, France; 1991.
5. Papirmeister B, Gross CL, Meier HL, Petrali JP, Johnson JB. Molecular basis for mustard-induced vesication. Toxocol Sci 1985;5(6 Pt 2):134-49.
6. Amir A, Chapman S, Gozes Y, Sahar R, Allon N. Protection by extracellular glutathione against sulfur mustard induced toxicity in vitro. Hum Exp Toxicol 1998;17(12):652-60.
7. Kadar T, Turetz J, Fishbine E, Sahar R, Chapman S, Amir A. Characterization of acute and delayed ocular lesions induced by sulfur mustard in rabbits. Curr Eye Res 2001;22(1):42-53.
8. Petrali JP, Dick EJ, Brozetti JJ, Hamilton TA, Finger AV. Acute ocular effects of mustard gas: ultrastructural pathology and immunohistopathology of exposed rabbit cornea. J Appl Toxicol 2000;20(1 Suppl):S173-5.

9. Amir A, Turetz J, Chapman S, Fishbeine E, Meshulam J, Sahar R, et al. Beneficial effects of topical anti-inflammatory drugs against sulfur mustard-induced ocular lesions in rabbits. J Appl Toxicol 2000;20(1 Suppl):S109-14.
10. Kulkarni PS. Steroids in ocular therapy. In: Zimmerman TJ, Kooner K, Sharir M, Fechtner RD, editor. Textbook of ocular pharmacology. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 1997. p. 61-74.
11. Fiévez L, Kirschvink N, Zhang WH, Lagente V, Lekeux P, Bureau F, et al. Effects of betamethasone on inflammation and emphysema induced by cadmium nebulisation in rats. Eur J Pharmacol 2009;606(1-3):210-4.