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Effect of tear deficiency on the course of endotoxin-induced keratitis

T.B. Gaydamaka,[1]

S.Ya. Rafalyuk[2]

[1]Filatov Eye Disease and Tissue Therapy Institute

[2]Danylo Halytsky Lviv National Medical University

Odessa, Lviv (Ukraine)

E-mail: drgaydamaka@list.ru

Background: *In recent years, the problem of tear production disorders and dry eye syndrome (DES) has become increasingly important because of significant worldwide prevalence of DES.*

Purpose: *To investigate the effect of decreased tear production on the course of endotoxin-induced keratitis in rabbits.*

Materials and Methods: *Animals were divided into three groups: group 1 (controls; 7 rabbits), group 2 (keratitis-only group; 9 rabbits, 18 eyes), and group 3 (keratitis + dry eye model group; 10 rabbits; 20 eyes). The state of the cornea was scored by Draize criteria (the degree of corneal opacity, degree of corneal edema, degree of corneal infiltration and corneal fluorescein staining area) at preselected intervals (24, 48 and 72 hours) postinfection.*

Results: *It should be noted that the analysis of clinical scores of corneal inflammation in the models of intrastromal endotoxin-induced keratitis in intact animals and in those with experimental dry eye showed that the clinical manifestations in the latter were more pronounced.*

Conclusion: *Tear deficiency significantly contributes to corneal inflammation in animal models of keratitis. Within the observation period, mean corneal edema, inflammatory infiltration and fluorescein staining scores in the (dry eye + keratitis) rabbit model were 21%, 21% and 17%, respectively higher than those in endotoxin-induced keratitis in animals without dry eye.*

Key words: endotoxin-induced keratitis, dry eye syndrome, animal models, tear deficiency, corneal inflammation, Draize criteria

Introduction

In recent years, the problem of tear production disorders and dry eye syndrome (DES) has become increasingly important because of significant worldwide prevalence of DES [1, 2]. The disease is found in 9-18% of the developed world's population, with a 4.5-fold increase in the detection rate over the last three decades. Moreover, DES has been found in almost every second first-time patient visiting the ophthalmologist for ocular disease or correction of vision. The syndrome incidence increases from 12% in the under-50s to 67% in the over-50s [3-6].

A number of pathochemical corneal alterations have been found in animal models of dry eye [7-9]. These alterations in particular involve elevated tear levels of lactate dehydrogenase and albumin, which are known to be the markers of corneal damage [10].

The increased incidence of dry eye in severe corneal inflammation and infections like keratitis is another reason for the special attention paid to the problem by ophthalmologists [11-16].

Previously, we have shown the greatly reduced reduction potential of corneal and tear fluid thiols in an animal model of dry eye [3]. In animals with dry eye, the development of keratitis results in a more abrupt and statistically significant decrease in reduced glutathione when compared with those exhibiting keratitis without dry eye. The presence of DES in the development of corneal inflammatory process results in increased oxidative stress evidenced by a statistically significant increase in oxidized glutathione both in the cornea and tear film [2, 17]. In this connection, investigation of the influence of tear deficiency on the corneal protective mechanisms in the presence of corneal inflammation is of significant interest.

The **purpose** of the study was to investigate the effect of decreased tear production on the course of endotoxin-induced keratitis in rabbits.

Materials and Methods

A total of 36 Chinchilla rabbits (weight, 2.2–2.9 kg) were used in the study.

All care and use of animals followed guidelines stipulated by the 2012 International Guiding Principles for Biomedical Research Involving Animals issued by the Council for the International Organizations of Medical Sciences.

Experimental keratitis was induced by intrastromal injection of 50 µL of 0.2% lipopolysaccharide (endotoxine) from Escherichia coli K235 in phosphate buffer saline (PBS) [18, 19].

0.1% benzalkonium chloride (BAC) in isotonic PBS (pH 7.3-7.4) was used to develop DES in the rabbit eye. Instillations were performed twice a day for 14 days [20, 21].

Animals were divided into three groups: group 1 (controls; 7 rabbits), group 2 (keratitis-only group; 9 rabbits, 18 eyes), and group 3 (keratitis + dry eye model group; 10 rabbits; 20 eyes).

The state of the cornea was scored by Draize criteria (the degree of corneal opacity, degree of corneal edema, degree of corneal infiltration and corneal fluorescein staining area) at preselected intervals (24, 48 and 72 hours) postinfection.

The signs of keratitis were scored based on the following scale: corneal edema (0 = no corneal edema and the whole cornea is transparent; 1 = local corneal epithelial edema at inflammation site; 2 = local epithelial edema with involvement of superficial stromal layers; 3 = local edema involving both superficial and deep stromal layers); inflammatory infiltration (0 = no infiltration; 1 = one to three punctate subepithelial infiltrates; 2 = multiple punctate subepithelial infiltrates (n > 3); 3 = multiple subepithelial infiltrates of 1 mm at least; 4 = local infiltration involving both superficial and deep stromal layers); corneal fluorescein staining (0 = no local staining; 1 = punctate corneal staining; 2 = less than 3 mm2 area; 3 = more than 3 mm2 area); corneal opacity (0 = no opacity; 1 = presence of opacity); and location of inflammation in the cornea (1 = central; 2 = paracentral).

Clinical data were analyzed with a non-parametric Mann-Whitney test using SPSS 11.0 software [22].

Results and Discussion

It should be noted that the mean Schirmer test values were 25% lower in eyes of dry eye model rabbits, which corresponded to the data reported by the authors of experimental dry eye model [20, 21].

Tables 1 to 3 summarize the results of the comparative analysis of the scores of the clinical signs of corneal pathology in eyes of dry eye model and keratitis model rabbits at different time points of the study.

Table 1 shows that, at the first time point, the corneal edema score and mean rank in rabbits affected with keratitis only were (1.78±0.55) and 17.97, respectively, vs. (1.95±0.51) and 20.88, respectively, in those affected with keratitis and dry eye. At the next time point, the corneal edema score and mean rank in rabbits affected with keratitis only were (2.44±0.51) and 14.44, respectively, vs. (2.95±0.22) and 24.05 ($P < 0.001$), respectively, in those affected with keratitis and dry eye. At the third time point, the corneal edema score and mean rank in rabbits affected with keratitis only were (1.56±0.62) and 16.94, respectively, vs. (1.80±0.41) and 21.80, respectively, in those affected with keratitis and dry eye.

Table 1. Corneal edema scores in the keratitis-only rabbit model and (dry eye + keratitis) rabbit model at different postinfection time points (points scored)

Postinfection time points	Statistic indices	Experimental conditions	
		Keratitis n = 18	Keratitis + dry eye n = 20
First time point (24 h)	M SD %	1.78 0.55 100.0	1.95 0.51 109.6
Second time point (48 h)	M SD %	2.44 0.51 100.0	2.95 0.22 120.9
Third time point (72 h)	M SD %	1.56 0.62 100.0	1.80 0.41 115.4

Note: %, the corneal edema score in the (dry eye + keratitis) group expressed as a percentage of that in the keratitis-only group; n, number of eyes

Table 2 demonstrates that, at 24h after exposure, the inflammatory infiltration score and mean rank in rabbits affected with keratitis only were (2.33±0.59) and 16.89, respectively, vs. (2.70±0.80) and 21.85, respectively, in those affected with keratitis and dry eye. At 48h after exposure, the inflammatory infiltration score and mean rank in rabbits affected with keratitis only were (3.06±0.38) and 13.81, respectively, vs. (3.70±0.57) and 24.63, respectively ($P < 0.001$), in those affected with keratitis and dry eye. At the last time point, the inflammatory infiltration score and mean rank in rabbits affected with keratitis only were (1.61±0.50) and 17.61, respectively, vs. (1.80±0.41) and 21.20, respectively, in those affected with keratitis and dry eye.

Table 3 shows that, at the first time point, the corneal fluorescein staining score and mean rank in rabbits affected with keratitis only were (1.78±0.55) and 18.44, respectively, vs. (1.90±0.55) and 20.45, respectively, in those affected with keratitis and dry eye. At the second time point, the corneal fluorescein staining score and mean rank in rabbits affected with keratitis only were (2.28±0.46) and 15.78, respectively, vs. (2.65±0.49) and 22.85 ($P < 0.05$), respectively, in those affected with keratitis and dry eye. At the last time pont, the corneal fluorescein staining score and mean rank in rabbits affected with keratitis only were (1.39±0.50) and 18.19, respectively, vs. (1.55±0.60) and 20.67 ($P < 0.05$), respectively, in (dry eye model + keratitis) rabbits.

Table 2. Inflammatory infiltration scores in the keratitis-only rabbit model and (dry eye + keratitis) rabbit model at different postinfection time points (points scored)

Postinfection time points	Statistic indices	Experimental conditions	
		Keratitis n = 18	Keratitis + dry eye n = 20
First time point (24 h)	M	2.33	2.70
	SD	0.59	0.80
	%	100.0	115.9
Second time point (48 h)	M	3.06	3.70
	SD	0.38	0.57
	%	100.0	120.9
Third time point (72 h)	M	1.61	1.80
	SD	0.50	0.41
	%	100.0	111.8

Note: %, the inflammatory infiltration score in the (dry eye + keratitis) group expressed as a percentage of that in the keratitis-only group; n, number of eyes

Table 3. ***I***nflammatory infiltration scores in the keratitis-only rabbit model and (dry eye + keratitis) rabbit model at different postinfection time points (points scored)

Postinfection time points	Statistic indices	Experimental conditions	
		Keratitis n = 18	Keratitis + dry eye n = 20
First time point (24 h)	M	1.78	1.90
	SD	0.55	0.55
	%	100.0	106.7
Second time point (48 h)	M	2.28	2.65
	SD	0.46	0.49
	%	100.0	116.9
Third time point (72 h)	M	1.39	1.55
	SD	0.50	0.60
	%	100.0	111.5

Note: %, the inflammatory infiltration score in the (dry eye + keratitis) group expressed as a percentage of that in the keratitis-only group; n, number of eyes

It should be noted that the analysis of clinical scores of corneal inflammation in the models of intrastromal endotoxin-induced keratitis intact animals and in those with experimental dry eye showed that the clinical manifestations in the latter were more pronounced. Therefore, one can believe that tear deficiency results in significantly impaired corneal protection if the inflammatory process develops. It is of no doubt that the metabolic abnormalities that we have detected within the cornea of rabbits with experimental dry eye [2, 17] play an important role in the mechanism of such a pathogenic effect of tear deficiency.

Conclusion

Tear deficiency significantly contributes to corneal inflammation in animal models of keratitis. Within the observation period, mean corneal edema, inflammatory infiltration and fluorescein staining scores in the (dry eye + keratitis) rabbit model were 21%, 21% and 17%, respectively higher than those in endotoxin-induced keratitis in animals without dry eye.

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