Human corneal cells as an *in vitro* model for toxicological studies of topical ocular drugs.

Ting-Jun Fan*

Laboratory for Corneal Tissue Engineering, College of Marine Life Sciences, Ocean University of China, Shandong province, China

Accepted on September 11, 2017

Editorial

As a complex optical organ, the human eye is composed of the anterior segment (cornea, iris and lens) and the posterior segment (vitreous, retina, choroid and sclera) and provides us with a clear vision. Many minor ocular injuries and lesions may cause sight-threatening illness and even loss of vision without appropriate medical treatment [1]. Hence plenty of topical ocular drugs and eye drops have been invented for the medical treatments and remedies for various eye disorders or diseases: keratitis, iritis, cataract, retinitis and glaucoma.

The cornea is a transparent front part of the eye and contributes most of the eye's focusing power. Topical ocular drugs may have unexpected by-effects such as corneal irritation, irreversible corneal damage, and even complete functional destruction in cornea if administered excessively [2]. These corneal injuries can lead to the disruption of its barrier function and eventually diminution of vision [3,4]. Consequently, the eye irritation potential of all eye drops manufactured for medical purpose must be assessed strictly in order to assure the safety of a topical medication and reduce its side effects. Therefore, series of eye toxicity tests are mandatory to guarantee that all eye drops could meet suitable safety criteria [5].

Animal experimentation was the only possible and credible method in ocular drug toxicity assessment and correlative biological response evaluation previously. Nevertheless, legislation has been introduced to reduce animal testing in many developed countries and to promote alternative techniques in recent years. These techniques include *ex vivo* toxicity tests based on organotypic models and *in vitro* toxicity assays based on cultured cell models. However, since *ex vivo* testing models can only provide assessment data in relatively short-term periods, they are not suitable for testing drugs that produce effects over extended time frames [5]. In comparison with *in vivo* and *ex vivo* testing, *in vitro* testing models using cultured cells are advantageous as they are simple, rapid, cost-effective, and devoid of hormonal, immune and neural influences. Meanwhile, *in vitro* testing models can provide data quantification, test replication and automation in assessment and reveal underlying mechanism of toxicity at the cellular and molecular levels [5-8].

Although many topical drugs are frequently administered for clinical purpose, their side effects on the cornea remain uncertain. Since the outmost cornea of the eye is in direct contact with eye drops and vulnerable to the damage of topical drugs, cytotoxicity study using an *in vitro* model of human corneal cells is essential to evaluate the side effects of the drugs [6,8]. As the use of immortalized corneal cell lines, with altered gene expression patterns, does not always faithfully represent the inherent behaviors of corneal cells *in vivo*, non-transfected human corneal cell lines established from native human corneal tissues become more and more indispensable in toxicological studies *in vitro* [5,9]. Recently, several non-transfected human corneal cell lines from donated human corneal tissues of endothelium, stroma and epithelium have been successfully established in our laboratory, and make it possible to assess the cytotoxicity of ocular drugs to human cornea and their possible cellular and molecular toxic mechanisms as well [10-12]. With the non-transfected human corneal cell lines, the cytotoxicity of various topical ocular drugs including anti-glaucoma drugs [13-18], anti-inflammation drugs [8,19], drugs for mydriasis [20-22], and local anesthetics [23-31] has been assessed. It suggests that almost all of these drugs exhibit dose- and time-dependent toxicities to human corneal cells through the induction of cell cycle arrest and apoptosis. Death receptor-mediated and mitochondrion-dependent pro-apoptotic pathways are highlighted in our studies [8,15-18,20-23,25-29,31]. The cytotoxic effects of the tested topical drugs and associated pro-apoptotic mechanisms are summarized in Table 1.

Our findings provide new insights into the cytotoxic and pro-apoptotic mechanisms in topical drugs, and also references for their prospective therapeutic interventions in eye clinics [6,8]. Furthermore, the *in vitro* assessed cytotoxicity and/or apoptosis-inducing effect of betaxolol, clonidine, phenylephrine and proparacaine have been also well verified *in vivo* using cat models [14,18,22,31]. These findings suggest that the established *in vitro* model using non-transfected human corneal cells is a rapid and cost-effective method to screen for corneal toxicity of topical drugs and remind clinicians that topical drugs should be used with great caution in clinical situations [27].

In summary, *in vitro* model established using native human corneal cells is a viable and important cytotoxicity assay system for ocular drugs. However, *in vitro* model is lack of capability in mimicking the complexities and numerous physiological parameters in human cornea thus data collected from *in vitro* model cannot predict potential risks and clinical inferences directly without further *in vivo* experiments. Recently, *in vitro* constructed human corneal equivalents have been developed in order to provide a more substantial platform with inherent characteristics of the native cornea for ocular drugs assessment [32].

Table 1. *List of ophthalmic drugs assayed using in vitro models of non-transfected human corneal cells.*

Drug name	Clinical purpose	Tested cell line	Toxic mechanism	Pro-apoptotic pathway	Ref. No.
Timolol	Anti-glaucoma	ntHCE cells	Cell apoptosis	ND	13
Betaxolol	Anti-glaucoma	ntHCE cells	Cell apoptosis	ND	14
Pilocarpine	Anti-glaucoma	ntHCS cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	15
Carteolol	Anti-glaucoma	ntHCEP cells	Cell cycle arrest, apoptosis	mt-dependent	16
Latanoprost	Anti-glaucoma	ntHCS cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	17
Clonidine	Anti-glaucoma	ntHCEP cells	Cell cycle arrest, apoptosis	Fas/TNFR1-mediated, mt-dependent	18
Pranoprofen	Anti-inflammation	ntHCE cells	Cell apoptosis	ND	19
Ofloxacin	Anti-inflammation	ntHCEP cells	Cell cycle arrest, apoptosis	TNFR1-mediated, mt-dependent	8
Atropine	Mydriasis	ntHCEP cells	Cell cycle arrest, apoptosis	TNFR1-mediated, mt-dependent	20
		ntHCE cells	Cell cycle arrest, apoptosis	TNFR1-mediated, mt-dependent	21
Phenylephrine	Mydriasis	ntHCS cells	Cell cycle arrest, apoptosis	mt-dependent	22
Lidocaine	Local anesthetic	ntHCS cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	23
		ntHCEP cells	Cell apoptosis	ND	24
		ntHCE cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	25
Tetracaine	Local anesthetic	ntHCEP cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	26
Proparacaine	Local anesthetic	ntHCE cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	27
		ntHCS cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	28
		ntHCEP cells	Cell cycle arrest, apoptosis	Fas-mediated, mt-dependent	29
Oxybuprocaine	Local anesthetic	ntHCE cells	Cell apoptosis	ND	30
		ntHCEP cells	Cell cycle arrest, apoptosis	TNFR1-mediated, mt-dependent	31

Note: FAS: Factor-Associated Suicide. Mt: Mitochondrion. ND: Not Determined. ntHCE cells: Non-Transfected Human Corneal Endothelial Cells. ntHCEP cells: Non-Transfected Human Corneal Epithelial Cells. ntHCS cells: Non-Transfected Human Corneal Stromal Cells. TNFR1: Tumor Necrosis Factor Receptor 1.

References

1. Ali MA, Klyne MA. Vision in Vertebrates. New York: Plenum Press 1985; pp:174-75.
2. McGee HT, Fraunfelder FW. Excessive use means several times a day during several days or even weeks. Toxicities of topical ophthalmic anesthetics. Expert Opin Drug Saf 2007;6:637-40.
3. Meek KM, Knupp C. Corneal structure and transparency. Prog Retin Eye Res 2015;49:1-16.
4. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ 2001;79:214-21.
5. Wilson SL, Ahearne M, Hopkinson A. An overview of current techniques for ocular toxicity testing. Toxicology 2015;327:32-46.
6. Lapalus P, Ettaiche M, Fredj-Reygrobellet D, et al. Cytotoxicity studies in ophthalmology. Lens Eye Toxic Res 1990;7:231-42.
7. Davila JC, Rodriguez RJ, Melchert RB, et al. Predictive value of *in vitro* model systems in toxicology. Annu Rev Pharmacol Toxicol 1998;38:63-96.
8. Fan WY, Wang DP, Wen Q, et al. Cytotoxicity of ofloxacin to human corneal epithelial cells by inducing cell cycle arrest and mitochondrion dependent apoptosis. EC Ophthalmol 2015;1:24-34.
9. Saarinen-Savolainen P, Järvinen T, Araki-Sasaki K, et al. Evaluation of cytotoxicity of various ophthalmic drugs, eye drop excipients and cyclodextrins in an immortalized human corneal epithelial cell line. Pharm Res. 1998;15:1275-80.
10. Fan T, Zhao J, Ma X, et al. Establishment of a continuous untransfected human corneal endothelial cell line and its biocompatibility to denuded amniotic membrane. Mol Vis 2011;17:469-80.
11. Fan TJ, Xu B, Zhao J, et al. Establishment of an untransfected human corneal epithelial cell line and its biocompatibility with denuded amniotic membrane. Int J Ophthalmol 2011;4:228-34.
12. Fan TJ, Hu XZ, Zhao J, et al. Establishment of an untransfected human corneal stromal cell line and its biocompatibility to acellular porcine corneal stroma. Int J Ophthalmol 2012;5:286-92.
13. Fan TJ, Sui BB, Wang QY, et al. Studies on the effect of timolol maleate on human corneal endothelial cells. Int Eye Sci 2013;13:1069-72.
14. Miao Y, Sun Q, Wen Q, et al. Cytotoxic effects of betaxolol on healthy corneal endothelial cells both *in vitro* and *in vivo*. Int J Ophthalmol 2014;7:14-21.

15. Yuan XL, Wen Q, Zhang MY, et al. Cytotoxicity of pilocarpine to human corneal stromal cells and its underlying cytotoxic mechanisms. Int J Ophthalmol 2016;9:505-11.

16. Shan M, Fan TJ. Cytotoxicity of carteolol to human corneal epithelial cells by inducing apoptosis via triggering the Bcl-2 family protein-mediated Mitochondrionl pro-apoptotic pathway. Toxicology *in Vitro* 2016;35:36-42.

17. Shen JW, Peng YY, Fan TJ. Cytotoxic efect of latanoprost on human corneal stromal cells *in vitro* and its possible mechanisms. Curr Eye Res 2017;42:534-41.

18. Fan D, Fan TJ. Clonidine induces apoptosis of human corneal epithelial cells through death receptors-mediated, Mitochondrion-dependent signaling pathway. Toxicol Sci 2017;156:252-60.

19. Li YH, Wen Q, Fan TJ, et al. Dose dependent cytotoxicity of pranoprofen in cultured human corneal endothelial cells by inducing apoptosis. Drug Chem Toxicol 2015;38:16-21.

20. Tian CL, Wen Q, Fan TJ. Cytotoxicity of atropine to human corneal epithelial cells by inducing cell cycle arrest and mitochondrion-dependent apoptosis. Exp Toxicol Pathol 2015;67:517-24.

21. Wen Q, Fan TJ, Tian CL. Cytotoxicity of atropine to human corneal endothelial cells by inducing mitochondrion-dependent apoptosis. Exp Biol Med (Maywood) 2016;241:1457-65.

22. Zhao J, Qiu Y, Tian CL, et al. The cytotoxic and pro-apoptotic effects of phenylephrine on corneal stromal cells via a mitochondrion-dependent pathway both *in vitro* and *in vivo*. Exp Toxicol Pathol 2016;68:409-17.

23. Zhou X, Li YH, Yu HZ, et al. Local anesthetic lidocaine induces apoptosis in human corneal stromal cells *in vitro*. Int J Ophthalmol 2013;6:766-71.

24. Miao Y, Wang RX, Yu HZ, et al. Cytotoxic effects of lidocaine on human corneal epithelial cells and its underlying mechanisms. J Shandong Univ (Nat Sci) 2014;49:8-14.

25. Yu HZ, Li YH, Wang RX, et al. Cytotoxicity of lidocaine to human corneal endothelial cells *in vitro*. Basic Clin Pharmacol Toxicol 2014;114:352-59.

26. Pang X, Fan TJ. The cytotoxic effect and possible mechanisms of tetracaine on human corneal epithelial cells *in vitro*. Int J Ophthalmol 2016;9:497-504.

27. Wen Q, Fan T, Bai S, et al. Cytotoxicity of proparacaine to human corneal endothelial cells *in vitro*. J Toxicol Sci 2015;40:427-36.

28. Fan WY, Sui YL, Fan TJ. Proparacaine induces cytotoxicity and Mitochondrion-dependent apoptosis in corneal stromal cells both *in vitro* and *in vivo*. Toxicol Res 2016;5:1434-44.

29. Bai S, Fan TJ. Cytotoxic effect of proparacaine on human corneal epithelial cells and its possible mechanisms. Mol Cell Toxicol 2017;13:29-36.

30. Ting-Jun Fan, Qian Wen, Miao-Miao Yu HZ, et al., Experimental studies on the effect of oxybuprocaine hydrochloride on human corneal endothelial cells. Int Eye Sci 2012;12:1442-46.

31. Fan WY, Wang DP, Wen Q, et al. The cytotoxic effect of oxybuprocaine on human corneal epithelial cells by inducing cell cycle arrest and mitochondria-dependent apoptosis. Hum Exp Toxicol 2017;36:765-75.

32. Katoh M, Hamajima F, Ogasawara T, et al. Establishment of a new *in vitro* test method for evaluation of eye irritancy using a reconstructed human corneal epithelial model, LabCyte CORNEA-MODEL. Toxicol *In Vitro* 2013;27:2184-92.

***Correspondence to:**

Ting-Jun Fan
Laboratory for Corneal Tissue Engineering
College of Marine Life Sciences
Ocean University of China
P.R. China
Tel: +86 532 82031637
E-mail: tjfan@ouc.edu.cn