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

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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 correlating 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 cytotoxicity 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].
References


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

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Clinical purpose</th>
<th>Tested cell line</th>
<th>Toxic mechanism</th>
<th>Pro-apoptotic pathway</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol</td>
<td>Anti-glaucoma</td>
<td>ntHCE cells</td>
<td>Cell apoptosis</td>
<td>ND</td>
<td>13</td>
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<tr>
<td>Betaxolol</td>
<td>Anti-glaucoma</td>
<td>ntHCE cells</td>
<td>Cell apoptosis</td>
<td>ND</td>
<td>14</td>
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<tr>
<td>Pilocarpine</td>
<td>Anti-glaucoma</td>
<td>ntHCS cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas-mediated, mt-dependent</td>
<td>15</td>
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<td>Carteolol</td>
<td>Anti-glaucoma</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>mt-dependent</td>
<td>16</td>
</tr>
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<td>Latanoprost</td>
<td>Anti-glaucoma</td>
<td>ntHCS cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas-mediated, mt-dependent</td>
<td>17</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Anti-glaucoma</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas/TNF-R1-mediated, mt-dependent</td>
<td>18</td>
</tr>
<tr>
<td>Pranoprofen</td>
<td>Anti-inflammation</td>
<td>ntHCE cells</td>
<td>Cell apoptosis</td>
<td>ND</td>
<td>19</td>
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<tr>
<td>Ofloxacin</td>
<td>Anti-inflammation</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>TNF-R1-mediated, mt-dependent</td>
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<tr>
<td>Atropine</td>
<td>Mydriasis</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>TNF-R1-mediated, mt-dependent</td>
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<tr>
<td>Phenylephrine</td>
<td>Mydriasis</td>
<td>ntHCS cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>mt-dependent</td>
<td>21</td>
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<tr>
<td>Lidocaine</td>
<td>Local anesthetic</td>
<td>ntHCS cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas-mediated, mt-dependent</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ntHCEP cells</td>
<td>Cell apoptosis</td>
<td>ND</td>
<td>23</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>Local anesthetic</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas-mediated, mt-dependent</td>
<td>24</td>
</tr>
<tr>
<td>Proparacaine</td>
<td>Local anesthetic</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>Fas-mediated, mt-dependent</td>
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<td>Oxybuprocaine</td>
<td>Local anesthetic</td>
<td>ntHCEP cells</td>
<td>Cell cycle arrest, apoptosis</td>
<td>TNF-R1-mediated, mt-dependent</td>
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</table>


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