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Perspective

Ocular Surface – Merging Challenges and Opportunities

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Dichotomies are double-edged: they can simplify and enlighten as well as exaggerate and entangle. Seeing the eye as anterior segment vs. posterior segment simplifies the formidable task of dissecting the function of the eye. Yet this view creates artificial divisions in a coherent whole. Clearly, vision requires the convergence of the light refractive function of the front of the eye with the light sensing function of the back of the eye. The National Eye Institute has long aimed to foster research across the visual pathway. Finding the right balance is a constant work in progress. A recently held scientific meeting which we co-organized with the United States Army Medical Research Institute of Chemical Defense, offered an opportunity to take stock of what the anterior segment in general, and the ocular surface in particular, bring to our understanding of biology and disease of the eye. Multiple dichotomies surfaced: acute vs. chronic disease; epithelial vs. endothelial damage; fibrotic vs. vascular pathology; inflammation vs. resolution response; chemical exposure vs. countermeasure; monotherapy vs. combination therapy; mechanistic vs. exploratory research; human vs. animal model. Merging some of these dichotomies is the goal of this paper.

Introduction

F. Scott Fitzgerald tells us that intelligence is the ability to hold two opposed ideas at the same time. Take the cornea. It has an extrinsic structural simplicity that masks an intrinsic mechanistic complexity. Simple and complex—intertwined. This duality emerges in many areas of anterior segment research. Case in point: ocular chemical toxicity—the theme of a recent transagency scientific meeting convened on February 25–26, 2020, in Bethesda, Maryland, by the United States National Institutes of Health (NIH) in partnership with the U.S. Army Medical Research Institute of Chemical Defense.[1] The meeting brought together subject matter experts from the civilian and military research communities to discuss the current state of the field in developing medical countermeasures (MCMs) to treat the acute and chronic effects of ocular chemical toxicity. This perspective highlights some themes that arose at the meeting.

Unlike skin, where the stratum corneum can prevent or slow the topical penetration of chemicals to protect the underlying tissues, the corneal epithelium lacks this particular protective property. Consequently, chemicals can more easily penetrate the ocular epithelia, thereby rendering the eyes particularly vulnerable to chemical-induced toxicity.[2] For example, compared to the skin, ocular toxicity induced by the chemical vesicant sulfur mustard, a strong bifunctional alkylating and blistering agent, typically manifests earlier and at lower concentrations—as low as one tenth.[3] Dissecting and counteracting the eye's vulnerability to chemical injury require traction in at least four domains: (1) Responses to corneal toxicity (acute and chronic); (2) Models of corneal toxic injury; (3) Mechanisms of corneal pathologies and wound healing, and (4) Therapies for corneal pathology. Crucially,

these domains impact the understanding of eye and corneal pathologies, beyond chemical injuries, including disease processes like corneal pain, neovascularization, inflammation and fibrosis. All these areas are at the core of the mission of the National Eye Institute (NEI/NIH).

Chemical injuries involving the eye account for 11% to 22% of ocular trauma.[4] Chemicals can be classified as acid, alkali, or neutral agents and pose a health and security threat not only to the military but also to the general public as well. The U.S. Department of Homeland Security (DHS) has identified almost 200 compounds as highly toxic chemicals (HTCs) of interest.[5] A number of these HTCs can be described as chemical vesicants and includes the previously mentioned sulfur mustard in addition to Lewisite and other arsenicals. The U.S. government is keenly interested in developing therapeutics and MCMs to treat ocular injuries resulting from exposure to these and other HTCs.

Through the Chemical Countermeasures Research Program (CCRP), implemented by the National Institute of Allergy and Infectious Diseases (NIAID/NIH) in 2006, the NIH has developed an expansive extramural research infrastructure dedicated to the discovery and early development of MCMs against those HTCs that DHS has identified as public health threats.[6] The CCRP infrastructure is comprised of interagency agreements with the Department of Defense and the Department of Health and Human Services, contract support resources, and a vast trans-NIH grant and cooperative agreement program called Countermeasures Against Chemical Threats (CounterACT). Under the longtime leadership of the NEI, the CCRP's ocular portfolio has included many projects that have contributed to the development of novel approaches to study ocular chemical injury. Excitedly, NEI-administered CounterACT projects dedicated to understanding and counteracting ocular chemical injury have also identified several promising therapeutic approaches and candidate MCMs, some of which are described here.

Responses to Corneal Toxicity

The breadth of the chemicals that can injure the eye represents a first level of complexity insofar the responses to such injuries, while overlapping, can be diverse. Even within the vesicant group, complexity of the corneal response to injury becomes apparent. For example, after sulfur mustard exposure, a common early response is photophobia followed by corneal erosions (as determined by fluorescein staining) and inflammation. These early/acute effects tend to resolve but then rebound as late/chronic effects, including corneal neovascularization, epithelial defects, chronic inflammation (especially conjunctivitis), and corneal opacity.[7]

Indeed, the so-called delayed-onset mustard gas keratopathy (MGK), which can occur any time between eight and 25 years after exposure, was observed in Iranian veterans from the Iraq-Iran war. In a retrospective study of 48 Iranian survivors, epithelial defects were seen in 31% of the patients, conjunctival vascular abnormalities in 50%, neovascularization in 71%, corneal scar or opacity in 87%, and chronic blepharitis and decreased tear meniscus in all subjects.[8]

These diverse corneal responses to chemical toxicity in turn impact the choice of appropriate model systems to experimentally model chemical exposure, injury progression, and evaluate potential countermeasures.

Models of Corneal Toxic Injury

The mammalian cornea is structurally conserved and consists of a basic three-layer plan: the stratified epithelium, stroma, and the single-layered endothelium. Bowman's membrane separates the epithelium from the stroma, whereas Descemet's membrane separates the stroma from the endothelium. Simple yet profound. Chemical toxicants, particularly vesicants, are known to affect each of the three main layers of the cornea. As such, there is a clear need for models with defined pathophysiology to further dissect the responses and underlying molecular mechanisms. Of course, the hope is that the simplicity of the model of choice does not obscure the true intricacy of the process being modeled; namely, chemical injury to the human eye.

Among the NEI-led CounterACT projects is the work of Eveleth and colleagues.[9] The research group recently reported on a corneal organ culture model in which vesicant-exposed (and control) rabbit corneas were cultured ex vivo in the appropriate media and placed on a rocker platform to allow the testing of various candidate interventions. This model subsequently enabled the group to demonstrate that a human fibroblast growth factor (FGF)–1 derivative, specifically TTHX1114, is protective against nitrogen mustard-induced damage of the corneal epithelium.[9] Similarly, Tewari-Singh and colleagues[10] used cultured human corneal epithelial cells to show that chloropicrin exposure (a chemical warfare agent now mainly used as

a soil pesticide) induces oxidative stress, DNA damage and lipid peroxidation.

Although such *ex vivo* models serve an important niche in advancing the fundamental knowledge of corneal chemical injury, *in vivo* animal models remain crucial in developing MCMs. Several animal model systems have been used to study ocular chemical exposure. Primary among these are the rat and rabbit model systems with the latter having been used more extensively to study the acute and chronic corneal toxicity after vesicant exposure. Using the rabbit acute corneal injury model, Agarwal and collaborators[11] successfully demonstrated that nitrogen mustard exposure induces conjunctival and eyelid swelling, as well as corneal opacity and ulceration in vivo. Additionally, both corneal thickness and epithelial degradation also acutely increased post-exposure and did not resolve until 28 days later.[11] Along with establishing a NM rabbit model, the group also published on a similar Lewisite rabbit model.[12]

Certainly, no model perfectly recapitulates the human cornea whether in biology or disease progression. Long gone are the days of attempting to transplant the rabbit cornea into the human eye.[13] Yet despite limitations, in vitro, ex vivo, and in vivo models have been and continue to be crucial to uncovering the underlying mechanisms of ocular chemical injury and evaluation of potential therapies.

Mechanisms of Corneal Pathologies and Wound Healing

The NIH has also funded a number of projects designed towards understanding the basic biology of ocular chemical responses. Such supported work includes metabolomics analysis by Vasiliou and colleagues[14] that showed that alterations in the sphingomyelin-ceramide pathway may contribute to the damaging effects of nitrogen mustard exposure. Although information on this pathway in the eye is quite limited, there is evidence of increased levels of apoptosis-related proteins in keratoconus. The similarity between keratoconus and nitrogen mustard exposure, especially epithelial thinning and corneal haze, is intriguing and raises possibilities of one area informing the other.

Similarly, results from the laboratory of Gordon et al. [15] showed that mustard activates matrix metalloproteinases (MMPs), which in turn degrade the extracellular matrix of the basement membrane and thereby induce separation of the epithelial layer from the stroma. Consequently, ongoing work is directed at analyzing the role of the MMP-inducer EMMPRIN in vesicant-induced epithelial-stromal separation of the cornea, as well as examining whether delayed wound healing in corneal wound beds after mustard injury is due to delayed deposition of fibronectin.

In addition to chemical-disease specific studies, the NIH has also supported screening efforts to identify potentially common mechanism(s) of corneal epithelial cell injury progression regardless of the particular chemical insult.[16] One such effort entails the use of high throughput screening using commercially available small interfering RNA (siRNA) libraries commonly used in the pharmaceutical industry to identify targets for drug discovery. Results from the siRNA screens enable ingenuity pathway analysis (IPA) to further understand the canonical pathways of the injury progression and the possibility of uncovering common signaling pathways among different toxicants.

Responses, models, and mechanisms of ocular injury lay the groundwork for the central goal of the NEI-led chemical toxicity effort; namely, developing effective medical countermeasures and therapies that buttress the natural defense mechanisms and blunt the pathologic outcomes. More complexity ensues.

Therapies for Corneal Pathology

Counteracting ocular chemical injuries is a central goal of the CCRP. Among the currently active NEI/CounterACT projects is that of Baker et al.[17] at Synedgen Inc. with the aim of evaluating the efficacy of new molecular entities from a polyglucosamine derivatives library following ocular sulfur mustard exposure in a rabbit model system. This class of polycationic glycopolymers targets the negatively charged glycosaminoglycans (GAGs) and mucins at epithelial surfaces, including the cornea's, and potentially reduces the activation of downstream inflammation and secondary damage.

In parallel, another recently awarded NEI project to Wollman utilizes high throughput, organ culture, image-based screening to identify compounds already approved by the Food and Drug Administration (FDA) that can mitigate corneal damage in response to vesicating agents.[18] More specifically, up to 770 such compounds will be screened for their ability to improve healing following nitrogen mustard exposure. Along with the advantage of the high throughput design—a distinct strength of the project is the possibility of repurposing an already approved drug thereby significantly accelerating potential development and regulatory approval.

Similarly, Mohan et al.,[19] from the University of Missouri at Columbia recently demonstrated that topical application of a novel multimodal non-steroidal topical ophthalmic formulation of four FDA-approved drugs, with differing mechanisms of action, was preliminarily efficacious in counteracting MGK in vivo. This is an innovative approach that uses combination therapy to simultaneously modulate COX-mediated inflammatory response; transforming growth factor β–induced corneal fibrosis; vascular endothelial growth factor (VEGF)-mediated corneal neovascularization; and corneal ulceration.

In addition to the previously described NIH-funded projects to repurpose FDA-approved compounds for MCM-focused indications, similar efforts are also in progress at the Israel Institute for Biological Research (IIBR). A particularly promising approach is the use of aflibercept (Eylea; an FDA-approved anti-VEGF medication for the treatment of the wet form of age-related macular degeneration and diabetic retinopathy) to mitigate sulfur mustard ocular injuries. IIBR researchers recently reported tantalizing data that a single injection of aflibercept after recovery of the acute phase of sulfur mustard exposure to the eye, resulted in significant reduction in ocular surface inflammation and corneal neovascularization. This work builds on the group's earlier findings that the anti-VEGF therapy bevacizumab (Avastin) was efficacious in reducing corneal neovascularization after sulfur mustard exposure.[20]

Conclusion

Pain, photophobia, corneal epithelial defects, keratitis, endothelial cell loss, edema, inflammatory response, conjunctivitis, tear disruption, neovascularization, corneal scarring, opacity, and blindness. A progression of acute corneal and ocular surface responses that frequently transition into chronic sequelae in response to ocular chemical toxicity. Clearly, the earlier the therapeutic intervention, the more likely that deleterious cascades can be aborted. It is tempting to think of these responses and mechanisms as linear cause-effect processes. This, in turn, makes it attractive to use the traditional reductionist approach to study these processes in relative isolation. One laboratory, one toxicant, one favorite response, one attractive pathway, one popular biomarker, one available model, one hopeful therapy. In a word, "silos." Of course, we know that neither biology nor pathobiology of the eye is linear. In the words of Ralph Waldo Emerson, "Cause and effect, means and ends, seed and fruit cannot be severed; for the effect already blooms in the cause, the end preexists in the means, the fruit in the seed." The deceptive simplicity of the anterior segment camouflages a microcosm of intricacy and complexity. And, hence, the opportunity. The future of anterior segment research, in general, and chemical toxicity, is propitious. The accessibility of the ocular surface, the breadth of the questions encountered, the availability of tools and resources for the detailed investigation of physiology and pathophysiology, position the anterior segment to capitalize on these opportunities through more collaborative work. Barriers among the different silos need to be minimized and bridges maximized. The apparent simplicity of the cornea and rest of the anterior segment invites it. The underlying complexity demands it.

Good progress has been made. A lot more remains to be done. Fitzgerald would sympathize.

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We invite scientists interested in further exploring NIH funding opportunities available to support discovery and early development of MCMs for ocular toxicity and research on the anterior segment of the eye to contact us.

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