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Clinical progression of ocular injury following arsenical vesicant lewisite exposure

Neera Tewari-Singh¹, Claire R. Croutch², Richard Tuttle², Dinesh G. Goswami¹, Rama Kant¹, Eric Peters², Tara Culley², David A. Ammar³, Robert W. Enzenauer³, J. Mark Petrash³, Robert P. Casillas², and Rajesh Agarwal¹

¹Department of Pharmaceutical Sciences, University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

²MRIGlobal, Kansas City, MO, USA

³Department of Ophthalmology, University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Abstract

Ocular injury by lewisite (LEW), a potential chemical warfare and terrorist agent, results in edema of eyelids, inflammation, massive corneal necrosis, and blindness. To enable screening of effective therapeutics to treat ocular injury from LEW, useful clinically-relevant endpoints are essential. Hence, we designed an efficient exposure system capable of exposing up to six New-Zealand white rabbits at one time, and assessed LEW vapor-induced progression of clinical ocular lesions mainly in the cornea. The right eye of each rabbit was exposed to LEW (0.2 mg/L) vapor for 2.5, 5.0, 7.5 and 10.0 min and clinical progression of injury was observed for 28 days post-exposure (dose-response study), or exposed to same LEW dose for 2.5 and 7.5 min and clinical progression of injury was observed for up to 56 days post-exposure (time-response study); left eye served as an unexposed control. Increasing LEW exposure caused corneal opacity within 6 h post-exposure, which increased up to 3 days, slightly reduced thereafter till 3 weeks, and again increased thereafter. LEW-induced corneal ulceration peaked at 1 day post-exposure and its increase thereafter was observed in phases. LEW exposure induced neovascularization starting at 7 days which peaked at 22–35 days post-exposure, and remained persistent thereafter. In addition, LEW exposure caused corneal thickness, iris redness, and redness and swelling of the conjunctiva. Together, these findings provide clinical sequelae of ocular injury following LEW exposure and for the first time establish clinically-relevant quantitative endpoints, to enable the further identification of histopathological and molecular events involved in LEW-induced ocular injury.

Address for correspondence: Rajesh Agarwal, Department of Pharmaceutical Sciences, University of Colorado Denver School of Pharmacy, Mail Stop C238, 12850 E. Montview Blvd., Room V20-2118, Aurora, CO 80045, USA. Tel: +1 3037244055. Fax: +1 3037247266. Rajesh.Agarwal@ucdenver.edu.

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Introduction

Lewisite [dichloro(2-chlorovinyl)arsine; L; LEW], a potential chemical warfare and terrorist agent, is a potent arsenical vesicant that induces rapid inception of severe pain and blistering upon exposure, and immediately causes skin, eye and respiratory tract damages (1-4). LEW was developed as an arsenical vesicant during World War I but was not used; however, stockpiles are known to exist posing a potential threat of its accidental exposure or use as a warfare/terrorist agent (5, 6). For example, the former Soviet Union stockpiled LEW as a weaponized mixture alongside the chemical warfare agent sulfur mustard [bis(2-chloroethyl) sulfide), SM] (7). Furthermore, LEW may have been used by the Japanese armed forces against China from 1937-1944 and by Iraq against Iranian soldiers in the Gulf war (1, 8). Based on studies of the related vesicant SM, eyes are the most sensitive organ to vesicant exposure with devastating short-and long-term injuries affecting up to 90% of the exposed individuals (9-12). Compared to other vesicants, relatively faster absorption of LEW can cause immediate pain and ocular injury, which can be severe and produce edema of eyelids, inflammation, massive corneal necrosis and blindness (13, 14). Literature reports on animal testing for ocular injuries are limited to the 1940's (15-18), whereby, LEW injuries were characterized by an immediate appearance of edema and blepharospasm, which can be serious with massive necrosis and eventual blindness (15, 18). Since LEW is also an organic trivalent arsenical compound that reacts with biological sulfhydryls, it causes injuries similar to other arsenic containing chemicals (1, 2). The main reactive and toxic breakdown product of LEW is arsine oxide; however, LEW also liberates hydrochloric acid, which lowers the pH of the eye and causes superficial opacity (16). These early reports on LEW-induced clinical and pathologic characteristics of corneal tissue give some information and description of injury; nonetheless, ocular injuries from this vesicant are not well defined. In addition, quantitative assessments to establish useful biomarkers for both mechanistic and efficacy studies are not available.

Even though human exposure to vesicating agents like SM and LEW can cause devastating ocular injuries, pain and panic, and blindness, there has been limited research to identify and validate effective therapies that can be deployed on a large scale to minimize pain and interrupt permanent ocular tissue damage resulting from an accidental or deliberate vesicant-exposure. More importantly, compared to SM whose effects have been studied extensively world-wide for over 100 years, efforts to study LEW-induced ocular injuries and subsequently to identify effective therapies for them are limited or undocumented. This is mainly because of an earlier reported antidote, dimercaprol (British Anti-Lewisite, BAL) and some of its recently tested chelators; however, their therapeutic efficacy is limited by inherent toxicity, narrow therapeutic window, and difficult administration, especially for ocular tissue treatment (2, 16, 19, 20). In view of these limitations, the present study assessed comprehensively the LEW vapor induced clinical lesions, in both dose- and time-dependent manner, and the progression of lesions starting 6 h post-LEW exposure to 56 days. Notably, we designed a unique ocular exposure system where up to six rabbits can be exposed simultaneously to the identical and controlled LEW vapor (right eye) for more consistent injury among groups and eliminating variation in the exposures. The study outcomes here, for the first time, show clinical manifestation of early and delayed symptoms

of ocular injury following LEW vapor exposure, and establish clinically-applicable quantitative ocular injury endpoints in a relevant injury model for the development of effective therapies.

Materials and Methods

LEW vapor exposure system design

The ocular exposure system for LEW vapor was designed to expose up to six rabbits simultaneously to the same controlled and measured chemical vesicant vapor challenge, with the ability to adjust both time of exposure and chemical vesicant dose (Fig. 1 A). This unique system allows for matched control (left eye) exposed to dilution air and challenge (right eye) animals to be simultaneously exposed to LEW, eliminating variation between exposures (Fig. 1B). A detailed diagram of the ocular exposure system is shown in Supplementary Fig. 1. Exposures were conducted with modified goggles that have a flow through design. Flow rates of challenge chemical agent vapor were controlled with critical stainless steel orifice control meters (Lenox Laser, Glen Arm, MD), that were laser drilled identically with 280um holes to assure that all flow rates were equilibrated through to the six sets of goggles. The orifices were flow calibrated for system exhaust flow rates in relation to flow control vacuum pressure to assure an identical LEW vapor flow, which was directed and distributed equally through each set of goggles from the total delivered vapor challenge. The control air exposure exhaust flows at each of the six locations were also equipped with critical orifice flow meters to provide an equal flow distribution of clean air to each control eye site. The exposure system incorporated a set of heat controlled diffusion cells for the generation of chemical vesicant vapor at controlled concentrations. Additional purified carbon and HEPA-filtered dry dilution air was introduced and challenge chemical vapor flow rates were controlled with calibrated mass flow controllers over the diffusion tubes to achieve the target exposure concentrations. The exposure system operates in a dynamic push/pull fashion with system pressure maintained at ambient pressure conditions and monitored with a differential pressure gauge. Critical flows were controlled and monitored at sub-critical flow conditions with a 0-30 inch Hg vacuum gauge. Exhaust flows were supplied with a Gas vacuum pump equipped with flow control valves. The dynamic system operation was used to supply fresh vapor challenge into the exposure goggles at sufficient flows to negate ocular damage related to flow velocity and to achieve continually replenished fresh chemical vesicant vapor challenge. The exposure goggles were made of molded silicone rubber with polycarbonate lenses and inside each goggle there was a butyl dam that sealed the eye into the goggle to create the exposure area. In addition, this is a flowing system (not static), with the LEW flow directed from Teflon tubing down onto the eye before it leaves the goggle via the exhaust. Therefore, we do not expect any LEW reaction to occur or any by-products to reach the eye; however, even if any LEW/material reaction did occur, it would be unlikely that any byproducts would come into contact with the eyes.

The exposure chambers were designed to fit the rabbit's head through a sealed dam with each animal's head fitted in a primary containment box to avoid exposure of the animal outside of the goggles and the head (Fig. 1B). The containment boxes were equipped with

carbon filtration units to capture any potential release of vapor as a primary safety feature of the system. All exposures within the boxes were conducted within the chemical agent (CA) hood line, in compliance with MRIGlobal standard operating procedures (SOP's) and safety procedures.

LEW system characterization

A diffusion tube was used for the generation and delivery of the vesicant LEW (14). All vapor transfer lines and valves of the system were composed of either Teflon® or polyethylethylketone (PEEK). These inert materials were used to generate a stable concentration vapor stream due to the high reactivity of LEW. Several of the transfer lines and valves were heated prior to the six-port split to ensure the LEW remained in the vapor phase. The entire system was designed using the shortest length of vapor delivery tubing as possible to reduce reactivity and condensation losses.

For all LEW analyses, a set of six 6-mm diameter Tenax TA solid sorbent tubes (SST) samples were collected, including pre- and post-exposure samples on days when animals were exposed. After sample collection, ranging from 2.5 to 10 min exposures at 300 ml/min flow, each SST was extracted by gravity using 2 mL of hexane followed by 1 mL of 9% hydrogen peroxide (H₂O₂) in methanol. The peroxide wash converts the LEW present on the Tenax TA to 2-chloro-vinylarsenic (V) acid (Cl-CH=CH-As(=O)(OH)₂, CVAOA) for total arsenic analysis by ICPMS. The peroxide/hexane extract was then concentrated to ~ 0.5 mL by nitrogen evaporation and brought to a final volume of 10 mL in a class A volumetric flask with 1% nitric acid in water. Aliquots from the nitric acid final volume mixture were removed and diluted followed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) analysis for total arsenic. The total arsenic found is directly (1:1 molar ratio) related to the LEW collected on the SST. On three individual days, LEW vapor was sampled from each vapor delivery exposure tube, and the average of the analysis of all six SSTs collected for an exposure challenge per exposure site was calculated. On the three individual exposure days, the percentage of pre- and post-exposure target concentrations (compared to control of 0.0207 mg/L) obtained for LEW were: 100% (pre) and 98% (post) at Day 1; 115% (pre) and 106% (post) at Day 2; 105% (pre) and 110% (post) at Day 3. Results showed reliability and reproducibility of the system to achieve a target LEW concentration (which did not go below 98% and above 110% of the target concentration) for subsequent animal ocular exposures.

Exposure procedure and clinical assessments

Following an approved protocol by the MRIGlobal IACUC, New Zealand white rabbits were procured from Charles River Laboratories (2.5 to 4.0 kg and not less than 3 months of age at time of arrival). After a quarantine and acclimation period, animals were randomized by weight into exposure groups for LEW exposure on study day 0, and clinical observations were taken from 3-10 eyes. Rabbits were administered buprenorphine HCl IM (0.05-0.1mg/kg) prior to challenge to aid in the management of pain. Approximately 30-60 min later, rabbits were anesthetized with a subcutaneous administration of ketamine (40 mg/kg) and xylazine (4 mg/kg). Once anesthetized, the eyes were taped open for exposing to comparable concentrations of LEW vapor, and goggles were secured around the animal's head for vapor exposure. The animals were placed on top of an absorptive pad and

positioned on one of the system's six built-in shelf units within the chemical hood. The right eye was exposed to LEW vapor; at the same time, the left eye was exposed to dilution air (no LEW) alone. After the target LEW vapor exposure was complete, the goggles were removed, and both the eyes (exposed to dilution air or LEW) were gently rinsed with saline (commercially available contact lens solution) to decontaminate the exposed area. Once the animal's eyes were gently washed and the rabbit deemed safe to remove from the hood line, it was placed in a clean container for transport to a post-exposure animal room and provided food and water *ad libitum*. Approximately 6 h post-LEW exposure, rabbits were administered buprenorphine SR (BupSR) once every 72 h and lights were dimmed in the animal room until otherwise directed by the veterinarian for further pain management. Clinical observations were performed daily throughout the study and included assessment of pain/discomfort signs including: hunched posture, altered activity level, tearing, grinding of teeth, covering of eyes with front feet, and menace response. Corneal injury was clinically evaluated prior to LEW exposure, then after 0.2 mg/L LEW exposure for 2.5, 5.0, 7.5 and 10 min at day 0 (6 h), day 1, day 3, day 7, day 14, day 22 and day 28 post-exposure in dose-response study and after 0.2 mg/L LEW exposure for 2.5 (lower dose) and 7.5 (higher dose) min at day 1, day 3, day 7, day 14, day 21, day 28, day 35, day 42, day 49 and day 56 post-exposure in time-response study. Whole eye injury was observed and digital pictures were taken at the above mentioned study time points after LEW and control exposures. Corneal injury assessments included corneal ulceration, corneal stromal injury, neovascularization, and eyelid notching (following the scoring system listed in Table 1), as well as pachymetry (TOMEY SP-3000 Pachymeter, Phoenix, AZ) to measure corneal thickness in both the control and LEW exposed eyes. Apart from corneal injury, percent animals with injury to the conjunctiva and iris were also recorded.

Statistical analysis

Clinical data were analyzed using one-way ANOVA and Tukey t-test for multiple comparisons (SigmaStat 2.03). Differences were considered significant if the p was ≤ 0.05 . Data are presented as mean $\pm$ standard error of mean (SEM).

Results

LEW exposure and ocular injury observations

Since sequelae of injury following LEW exposure is not well defined, the current study was designed to evaluate the clinical manifestation 6 h to 56 days (8 weeks) post LEW-exposure. Due to their size and similarity to human eye, New Zealand White rabbit eyes were exposed to LEW in a pre-pilot study to first determine its target dose employing the information from a historical literature reference which indicated that conjunctivitis and eyelid swelling would occur within 5.0 min of exposure (17). Results from the pilot study using the designed vapor exposure system showed that 0.2 mg/L LEW exposure for 10 min caused substantial and immediate (within 2 h of exposure) injury to the eye with severe inflammation, swelling of the eyelids and conjunctiva (resulting in the eye being closed), redness of iris, and ocular discharge, which hindered eye exam until 6-24 h post-exposure (data not shown). Corneal opacity was severe up to one week post-exposure (Fig. 2A) and persisted up two weeks post-exposure; neovascularization was also observed after 3 days post-exposure in this pilot study

(data not shown). Based on these pilot data, first a dose-response effect of LEW exposure was observed by exposing right eye of the rabbits to 0.2 mg/L LEW for 2.5, 5.0, 7.5 and 10.0 min, and the clinical manifestation of symptoms were observed up to 28 days post-exposure. LEW exposure caused red squinty eyes, eyelid swelling, conjunctivitis, ocular edema and inflammation, hunched posture, cloudy eyes, thick whitish/yellow ocular discharge, matted fur around the eye, and severe corneal injury, which increased in a dose-dependent manner (data not shown). To further examine both the acute and chronic effects of LEW exposure, 2.5 and 7.5 min exposure times were selected for a time-response study for up to 56 days post-exposure. All the clinical injury symptoms to the cornea, conjunctiva and iris, which developed on day 1-3 following 2.5 min LEW exposure, started resolving by 7 days post-exposure, and by 56 days post-exposure, eyes appeared comparable to controls (Fig. 2B). However, LEW exposure at the longer duration of 7.5 min, induced long-lasting ocular lesions including corneal opacity and neovascularization which persisted up to 56 day post-exposure (Fig. 2C). Two of the animals at this time point were euthanized due to severe ocular injury and perforation.

Assessment of the corneal injury following LEW exposure

Since vesicating agents primarily target the corneal tissue, based on the clinical observations herein and similar studies with SM, a more detailed evaluation of corneal injury parameters including opacity, ulceration, neovascularization, and thickness was carried out via slit lamp observations.

a) Corneal opacity—Corneal opacity was evaluated as the loss of transparency or clouding of the cornea and visibility of iris vessels and pupil (Table 1). LEW exposure caused a dose-dependent increase in corneal opacity within 6 h (day 0) post-exposure, which was maximal at day 3 post-exposure and decreased thereafter from 21 to 28 days post-exposure (Fig. 3A). Similar results were obtained for 2.5 and 7.5 min exposures for up to 28 days post-exposure in time-response study (Fig. 3B). At day 3 post-exposure, corneal opacity scores were 1.6, 2.1, 2.4 and 2.7 at 2.5, 5.0, 7.5 and 10 min of LEW exposure, respectively (Fig. 3A). In time-response study, exposure to LEW for 2.5 min resulted in a maximal opacity evident at 3 days post-exposure, which resolved to baseline levels by 21 days. However, opacity recurred and increased by 28 days post-exposure, which was significant up to 35 days post-exposure but remained elevated until the end of the observation period (Fig. 3B and C). Extending the LEW exposure time to 7.5 min resulted in severe corneal opacity beginning 6 h post-exposure, which was maximal at 3 days after exposure, and persisted until the end of the observation period at 56 days post-exposure (Fig. 3B and C).

b) Corneal ulceration—The uptake of fluorescein (green) stain by exposed stroma due to the disruption of epithelial layer was used to measure this epithelial lesion (blue arrows, Fig. 4C). Results showed that corneal ulceration peaked 1 day post-exposure at all the exposure times and resolved thereafter in both the dose- and time-response studies (Fig. 4A and B). Similar to the dose-response study, corneal ulceration was observed in rabbit eyes exposed to LEW for 2.5 or 7.5 min at day 1 post-exposure in time-response study (Fig. 4B). In 7.5 min LEW exposed eyes, corneal ulceration recurred from day 14 to day 35 post-exposure

and again at 56 day post-exposure (Fig. 4B and C). However, in the shorter exposure duration of 2.5 min, corneal ulceration started to resolve after 3 days of exposure, which was again significant at 42 days post-exposure and by 56 day post-exposure this lesion completely resolved (Fig. 4B and C).

c) Corneal neovascularization—Neovascularization was quantified as the percent length of the blood vessel growth in the cornea, which is presented here as the average of neovascularization from four quadrants of the cornea (Table 1). Corneal neovascularization was observed in eyes exposed to LEW for 2.5-10 min durations starting 3 days post-exposure; however, a higher score of over 2 (longest vessel length was approximately over 50% of the radius of the cornea) was observed in 5.0-10 min LEW exposed corneas up to 28 days post-exposure (Fig. 5A). Comparable LEW-induced corneal neovascularization was observed in both the dose- and time-response studies for up to 28 days post-exposure (Fig. 5A and B). In the time-response study, LEW-induced neovascularization was observed 7 day post-exposure and peaked at 35 days after exposure in corneas exposed to both 2.5 and 7.5 min durations. However, longer exposure (7.5 min) to LEW resulted in a neovascularization score of over 3 (longest vessel length up to approximately 51-75% of the radius of the cornea), which persisted thereafter till the end of the study up to 56 days post-exposure (Fig. 5B and C).

d) Corneal thickness—Based on the dose-response study results and observations, pachymetry readings were recorded to measure the corneal thickness (related to edema). A significant increase in corneal thickness was recorded starting at day 1 post LEW exposure, which was maximal at 3 day post 7.5 min exposure and at 7 day post 2.5 min exposure (Fig. 6). At 3 day post LEW-exposure, where compared to average control corneal thickness of 355.95 μm , 7.5 min exposed corneas were 959.17 μm thick (Fig. 6). Following 2.5 min LEW exposure, average corneal thickness was 980.50 μm compared to 368.40 μm corneal thickness in the control group at 7 day post-exposure (Fig. 6). The corneal thickness decreased to 460.50 μm at day 56 post 2.5 min LEW exposure; however, following a longer duration (7.5 min) of LEW exposure, there was a decrease in corneal thickness at 42 day post-exposure, which again elevated to 713.00 μm at day 56 post-exposure (Fig 6).

Injury to the conjunctiva and iris following LEW exposure

a) Redness and swelling of the conjunctiva—Within 6 h (Day 0) of LEW exposure for 5.0-10 min durations, all the animals showed severe conjunctival swelling in both dose- and time-response studies (Fig. 7A and B). The observations showed that the conjunctiva almost covered the eyes with moonlike appearance, and animals could not completely open their eyes (Fig. 7 E). LEW-induced swelling in the conjunctiva decreased 3-14 days post-exposure and resolved thereafter (Fig. 7A and B). Similarly, LEW exposure resulted in severe redness of the conjunctiva which peaked at 1 day post-exposure and then resolved in all the animals by 4-7 days post-exposure (Fig. 7 C-E). However, exposure at shorter duration (2.5 min) maximal 70% of animals showed this increase 1 day post- LEW exposure in dose-response study which was present in even fewer animals in time-response study (Fig. 7C and D).

b) Redness of the iris—LEW exposure caused an increase in percent animals with LEW-induced redness of the iris, which was observed within 6 h (Day 0) post-exposure, and animals with this effect were maximal at 1 day post-exposure (Fig. 8A-C). In comparison to the dose-response study, where 2.5 min and 7.5 min LEW exposures had lower and variable percent of animals showing iris redness at day 1-3 post-exposure, both 2.5 and 7.5 min exposures in time-response study, resulted in 100% animals with redness in the iris (Fig. 8B). The redness of the iris in both the dose- and time-response studies resolved in all the animals by day 7 post-exposure (Fig. 8A-C).

Discussion

The present study characterizes LEW-induced progression of clinical lesions following ocular vapor exposure in a rabbit model using a more consistent and efficient exposure system. The outcomes here provide valuable quantitative biomarkers of clinical relevance for assessing efficacy of treatments to mitigate ocular injury from LEW exposure. Furthermore, the clinical features of exposure to LEW may also be useful in diagnosis, as well as for further assessment of the histopathological lesions and related ocular toxicity pathways. This study is also highly significant because data on ocular toxicity from LEW are scarce, and possible ocular use of the skin antidote BAL has substantial drawbacks due to its inherent toxicity and limited window as well as route of treatment (2, 16, 19, 20).

LEW causes injury to skin and eyes immediately upon exposure in contrast to SM that has a latent phase and delayed symptoms; though, both are strong vesicating agents (1). LEW exposure in humans results in swelling and edema, inflammation to the iris and conjunctiva as well as corneal damage (14); the injuries which were also observed in the current study in rabbits after LEW exposure suggesting this as a relevant ocular injury model for LEW. As reported earlier by Mann et al., the current study also documents an immediate and early swelling and redness on eyelids, conjunctiva and iris of the eye, and edematous and opaque cornea at all the LEW exposure times (15). Studies with SM have shown that corneal ulceration peaks at 1-2 days post-exposure and then decreases by 4 days post-exposure (10, 21). Following LEW exposure in the present study, corneal ulceration also peaked at 1 day post-exposure; however, after decreasing till 7 day post-exposure, corneal ulceration recurred at 14 day post-LEW exposure. Also, similar to earlier report with SM (22), neovascularization was a later consequence which was visible at 7 day post LEW exposure, increased thereafter to 28 days and then remained persistent till the end of the observation time. The persistent neovascularization which remained elevated at 56 day post-exposure indicates oxygen deprivation as well as persistent inflammation and injury to the cornea causing loss of transparency and vision problems. Edema and corneal thickness are also reported following SM exposure, which peaked by 7 days post-exposure (10, 22-24), and consistent with that, corneal thickness was maximal at 1-7 days post LEW exposure indicating edema and inflammation. LEW at both 2.5 and 7.5 min exposure durations caused swelling and redness of the conjunctiva and iris, which resolved simultaneously at 4-14 days post-exposure; these injuries have been reported with both LEW and SM (10, 15, 22). Importantly, though the gross features of LEW-induced ocular injury in the present study in rabbits were observed within 6 h post-exposure, these lesions in rabbits including corneal injuries were observed 24-48 h post SM exposure (21, 25). However, similar to SM

exposure, the severity of the clinical lesions including conjunctival edema, corneal opacity, corneal-stromal injury with LEW also peaked 24-72 h post-exposure (23, 26). In reported SM studies, clinical injury resolved within 1-2 weeks, depending on the dose of SM; however, a second phase of injury elevation characterized by corneal edema, opacity, recurrent erosions and neovascularization was observed around two –five weeks post exposure (21, 23-27). Following LEW exposure, as detailed above caused a complete healing of injury to the conjunctiva and iris within 2 weeks of exposure but corneal injury recurred in phases 1-3 weeks post-exposure (delayed phase of injury).

Overall, gross injuries following ocular exposure of LEW are dose-dependent and could be similar and/or comparable to those reported for SM. Since corneal wounding, ulceration, inflammation, neovascularization and their progression were observed clinically and quantified with LEW in this study, examination of related histopathology and mechanisms of acute and long-term corneal injury and healing are now warranted in future. In this regard, it is reported that LEW and SM produce lesions that have similar gross appearance in skin, eye and lungs, but their pathology and development can differ since the mechanism of action of LEW-induced injuries is also associated with arsenic poisoning and inhibition of carbohydrate metabolism (3, 5, 19, 28). Hence, deciphering the mechanism of ocular injuries following LEW exposure would help in outlining the pathways that could be targeted to develop effective therapies against LEW-induced ocular injuries.

Conclusions

The study on the progression of ocular injury with LEW in this rabbit model provides useful clinical end points for efficacy studies and lays the foundation for further in-depth investigation of the histopathological and molecular events that lead to clinical injury on the most affected corneal tissue. These additional studies would further develop this injury model providing an opportunity to compare the corneal injury from LEW with corneal injuries reported with other vesicating agents, and more importantly for testing targeted treatments against the severe corneal injury from this potent arsenical vesicating agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. LEW vapor system for ocular exposure with the capacity to expose up to six rabbits simultaneously

Exposure system setup under the safety cabinet (A), and LEW ocular exposure of rabbits (B).

Figure 2. Clinical progression of ocular injury following LEW exposure

New Zealand white rabbits (n=5/group) were exposed to LEW (0.2 mg/L) vapor for either 10 min and clinical progression of injury was observed for 7 days post-exposure, or for 2.5 and 5.0 min and clinical progression of injury was observed for up to 56 days post-exposure as detailed in materials and methods. The right eye was exposed to LEW vapor and at the same time, the left eye was exposed to dilution air (no LEW) alone. Representative pictures show the injury to rabbit eyes from the highest (10 min) LEW exposure time at 1 and 7 days post-exposure (A) and the progression of ocular injury from 1 day to 56 days following 2.5 (B) and 7.5 (C) min LEW exposure. Red arrows, Inflammation: swelling and edema in the eyelid and conjunctiva and iris redness; blue arrows, corneal-stromal injury (opacity); purple arrows, neovascularization

Figure 3. Effect of LEW vapor on corneal opacity

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for either 2.5, 5.0, 7.5 and 10 min and corneal opacity was evaluated from 6 h (Day 0) to 28 days post-exposure (A), or for 2.5 and 7.5 min and corneal opacity was evaluated from 1 day to 56 days post-exposure (B) as detailed in materials and methods. Representative slit-lamp pictures show corneal opacity in rabbit eyes from 1 day to 56 days following 2.5 and 7.5 min LEW exposure (C). Blue arrows, scarring or clouding of cornea or corneal opacity. Data presented are mean $\pm$ SEM (n=3-10). *, p<0.05 for 2.5 min LEW exposure; †, p<0.05 for 5.0 min LEW exposure; ‡, p<0.05 for 7.5 min LEW exposure; §, p<0.05 for 10.0 min LEW exposure.

Figure 4. Effect of LEW vapor on corneal ulceration

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for either 2.5, 5.0, 7.5 and 10 min and corneal ulceration was evaluated from 6 h (Day 0) to 28 days post-exposure (A), or for 2.5 and 7.5 min and corneal ulceration was evaluated from 1 day to 56 days post-exposure (B) as detailed in materials and methods. Representative slit-lamp pictures show corneal ulceration in rabbit eyes from 1 day to 56 days following 2.5 and 7.5 min LEW exposure (C). Blue arrows, corneal ulceration; fluorescein stain taken up by exposed stroma, due to the disruption of epithelial layer, appears green. Data presented are mean $\pm$ SEM (n=3-10). *, $p < 0.05$ for 2.5 min LEW exposure; †, $p < 0.05$ for 5.0 min LEW exposure; ‡, $p < 0.05$ for 7.5 min LEW exposure; §, $p < 0.05$ for 10.0 min LEW exposure.

Figure 5. Effect of LEW vapor on corneal neovascularization

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for either 2.5, 5.0, 7.5 and 10 min and corneal neovascularization was evaluated from 6 h (Day 0) to 28 days post-exposure (A), or for 2.5 and 7.5 min and corneal neovascularization was evaluated from 1 day to 56 days post-exposure (B) as detailed in materials and methods. Representative slit-lamp pictures show corneal neovascularization in rabbit eyes from 1 day to 56 days following 2.5 and 7.5 min LEW exposure (C). Blue arrows, neovascularization seen as excessive growth of blood vessels. Data presented are mean $\pm$ SEM (n=3-10). *, $p < 0.05$ for 2.5 min LEW exposure; †, $p < 0.05$ for 5.0 min LEW exposure; ‡, $p < 0.05$ for 7.5 min LEW exposure; §, $p < 0.05$ for 10.0 min LEW exposure.

Figure 6. Effect of LEW vapor on corneal thickness

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for 2.5 and 7.5 min and corneal thickness was measured using a pachymeter from 1 day to 56 days post-exposure as detailed in materials and methods. Data presented are mean $\pm$ SEM (n=3-10). *, $p < 0.05$ for 2.5 min LEW exposure; ‡, $p < 0.05$ for 7.5 min LEW exposure.

Figure 7. Effect of LEW vapor on the conjunctiva

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for either 2.5, 5.0, 7.5 and 10 min and percent animals with conjunctival swelling (A) and redness (C) were evaluated from 6 h (Day 0) to 28 days post-exposure or for 2.5 and 7.5 min and percent animals with conjunctival swelling (B) and redness (D) were evaluated from 1 day to 56 days post-exposure (B) as detailed in materials and methods. Representative slit-lamp pictures show redness and swelling in the conjunctiva of rabbit eyes from 1 day to 56 days following 2.5 and 7.5 min LEW exposure (n=3-10, E). Blue arrows, redness and swelling in the conjunctiva.

Figure 8. Effect of LEW vapor on the iris

New Zealand white rabbits were exposed to LEW (0.2 mg/L) vapor for either 2.5, 5.0, 7.5 and 10 min and percent animals with injury to the iris were evaluated from 6 h (Day 0) to 28 days post-exposure (A), or for 2.5 and 7.5 min and percent animals with injury to the iris were evaluated from 1 day to 56 days post-exposure (B) as detailed in materials and methods. Representative slit-lamp pictures show redness in the iris of rabbit eyes from 1 day to 56 days following 2.5 and 7.5 min LEW exposure (n=3-10, C). Blue arrows, redness and swelling in the conjunctiva.

Table 1
Scoring system for clinical study parameters

Clinical parameter	Score (Grade)	Definition
Corneal stromal Injury (opacity)	0	Cornea transparent
	1	Minimal loss of corneal transparency
	2	Moderate loss of corneal transparency- iris vessels and pupil still visible
	3	Severe loss of corneal transparency- either iris vessels or pupil not visible
	4	Diffuse loss of corneal transparency- neither iris vessels nor pupil visible
Neovascularization (NV)	0	No neovascularization present
	1	Longest vessel length up to approximately 25% of the radius of the cornea
	2	Longest vessel length up to approximately 26-51% of the radius of the cornea
	3	Longest vessel length up to approximately 51-75% of the radius of the cornea
	4	Longest vessel length greater than approximately 75% of the radius of the cornea
Corneal Ulceration	0	Corneal ulceration not present
	1	Corneal ulceration present