Lesions Caused by Ricin Applied to Rabbit Eyes

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Purpose. Ricin, a highly potent toxin from castor beans, is a potential biological weapon that could be dispersed in the air as dust or aerosol. In these forms, ricin, besides being inhaled, could reach unprotected eyes. The present research was performed to ascertain the lesions that the toxin causes when applied to rabbit eyes.

Methods. Ricin was applied to rabbit eyes in solution, in quantities ranging from 1 to 100 μg. Animals were observed until death, when eyes and internal organs were removed and fixed. Sections were stained and examined microscopically.

Results. Ricin caused inflammation of the eyes and adnexa, visible both macroscopically and histologically. The damage was greatly reduced by rinsing the eyes with 10% lactose, provided the rinsing was done almost immediately after application of the toxin. Rinsing with phosphate-buffered saline (PBS) had no effect. With the highest dosage, congestion of internal organs was also apparent.

Conclusions. Application of ricin to eyes causes local damage, mainly of the inflammatory type. The ineffectiveness of rapid rinsing with PBS and the partial efficacy of rapid rinsing with lactose indicate that the toxin quickly binds to and is taken up by cells. The lesions of internal organs show that ricin applied to the eyes can be absorbed, pass into the circulation, and, at least at some dosages, damage internal organs. (*Invest Ophthalmol Vis Sci.* 2005;46:1113–1116) DOI:10.1167/iovs.04-0769

Ricin, a protein from *Ricinus communis* seeds (castor beans) is a lectin with specificity for sugars with the galactose structure and is highly toxic to animals and humans. The toxin is a ribosome-inactivating protein (RIP)—that is, an enzyme that irreversibly damages ribosomes[1] by removing a single adenine (A_{4324}) from the rRNA 60S subunit,[2] thus arresting protein synthesis and causing cell death. Systemic administration of ricin causes severe apoptotic and necrotic lesions in several organs, particularly in the lymphoid organs, liver, kidney, and gut (reviewed in Ref. 3).

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Interest in ricin has grown mainly because its enzymic A chain is chemically linked to, or fused with, antibodies to form immunotoxins, which it is hoped will be useful in the treatment of cancer and other ailments (reviewed in Ref. 4).

Ricin is a potent poison easy to prepare from commonly obtainable material and has been used for suicidal and homicidal purposes. In the 1978 case known as "the murder with the umbrella," the death of Georgi Markov, a Bulgarian journalist living in London, was attributed to poisoning by ricin contained in the cavity of a small metal pellet, apparently shot from a weapon concealed in an umbrella.[5] Also, there are fears that ricin and similar toxins could be used as weapons in warfare or terrorist attacks if sprayed in the air as an aerosol or dust (review in Ref. 6). This concern prompted research to investigate the lesions brought about by,[7–8] and the protective effects of vaccines or antibodies against,[9–11] inhaled ricin.

It was remarked that should ricin, in the form of aerosol or dust suspended in the air, reach unprotected humans, it would be inhaled and also would come in contact with eyes.[12] Still, in the available literature there are no detailed descriptions of the possible damage ricin causes to eyes, except for the irritating effects of "ricin powder" on the eye observed by Paul Ehrlich in 1891 (quoted in Ref. 13) and the mention of eye inflammation in several instructions for possible adverse events. The present investigation was undertaken to ascertain the damage caused locally and systemically by ricin applied to the eye of the rabbit.

Methods

Animals and Treatment

White male rabbits (2.5 ± 0.2 kg; Charles River Laboratories Italia S.p.A., Calco, Italy) fed on a commercial diet were randomly assigned to have different doses of ricin applied to the eyes. Ricin was prepared from castor beans as described elsewhere[14] and was stored freeze-dried at −20°C. It was dissolved in phosphate-buffered saline (PBS), and 100 μL of solution containing various quantities (1, 10, 32, 56, or 100 μg) was applied to one eye, and the other eye was treated with PBS only, as a control. In some experiments, after the application of 10 μg ricin, the eyes were washed with PBS or with a 10% solution of lactose in PBS. The animals were observed periodically and were killed with 50 mg/kg pentobarbital 24 hours or 7 days after treatment, and the eyes and internal organs were removed. Unless otherwise stated, three animals per treatment were used, and the results were remarkably consistent. Experiments were performed according to the Italian Accreditation of Laboratory Animal Care, and in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, with an experimental protocol approved by the Ethics Committee of the University of Bologna and by the Italian Ministry of Health.

Histologic Procedure

The eyes, liver, kidney, lung, and spleen were removed and fixed in 10% neutral buffered formalin. The eyeballs were sectioned in the sagittal plane and subsequently embedded in paraffin for light micros-

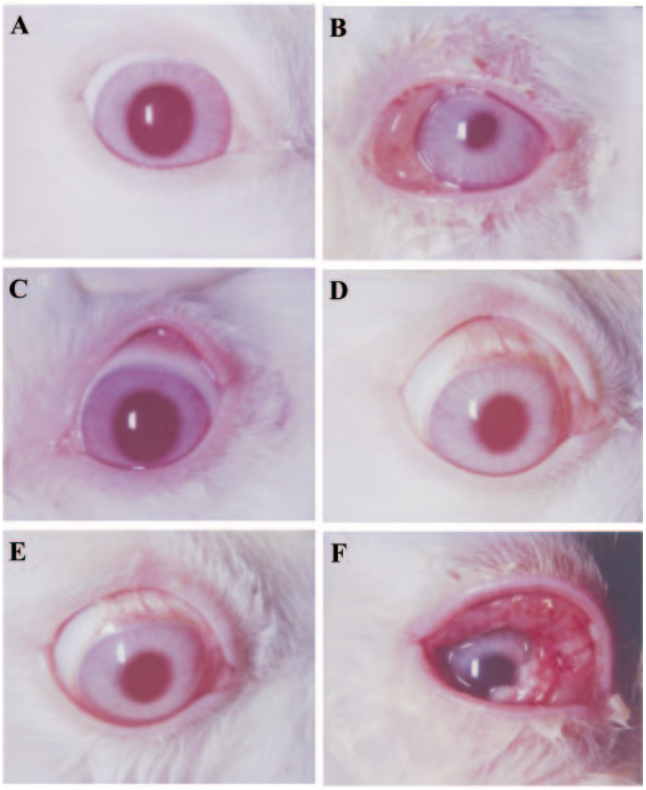

FIGURE 1. (**A**) Eye before treatment. (**B**) Eye 24 hours after 10 μg ricin, showing slight palpebral chemosis, conjunctival chemosis, and hyperemia of the conjunctiva; moderate discharge. (**C**) Eye 7 days after 10 μg ricin, showing no observable lesions. (**D**) Eye 4 hours after 100 μg ricin, showing no observable lesions. (**E**) Eye 8 hours after 100 μg ricin, showing moderate perilimbal conjunctival hyperemia. (**F**) Eye 24 hours after 100 μg ricin showing marked palpebral chemosis and marked conjunctival chemosis, hyperemia, and discharge.

copy. Serial sections of the central block, including the anterior segment, lens, and optic nerve, and the nasal and temporal calottes were stained with hematoxylin-eosin. The lachrymal gland and parts of the upper and lower lids and samples of internal organs were processed accordingly. Gomori's reticulin stain and periodic acid-Schiff were applied on sections of liver and kidney, respectively.

RESULTS

Topical application of ricin to rabbit eyes caused alterations that appeared with variable delay, depending on the dosage.

The lowest dose (1 μg) of ricin did not cause visible alteration of the eye. When 10 μg was applied, the eye appeared normal until 4 hours, and signs of inflammation—namely, hyperemia and secretion—appeared at 8 hours, becoming progressively more severe until a maximum was reached at 24 to 32 hours (Fig. 1B). The signs began to regress gradually at 48 and 72 hours, until the eye appeared almost normal at 7 days (Fig. 1C).

All doses of ricin higher than 10 μg caused a similar inflammatory reaction, which began to be visible at earlier times after application: 4 hours after 32 μg and 1 hour after 56 and 100 μg (results not shown). All rabbits receiving these amounts of toxin had to be anesthetized and killed by exsanguination after 24 hours, because of visible suffering. In two of three rabbits that received the highest dose of ricin (100 μg), besides a severe inflammation visible in the treated eye at 4, 8, and 24 hours (Figs. 1D–F, respectively), at the postmortem examination, the bladder wall appeared dark red, because of severe congestion. In these animals, some inflammation was visible in the contralateral eye, treated with an equal volume of PBS.

In some rabbits receiving 10 μg ricin, the eyes were rinsed in an attempt to prevent or at least limit the damage induced by the toxin (Fig. 2B). Rinsing with PBS did not have any beneficial effect, even when it was done 1 minute after application of the toxin (Fig. 2C). Rinsing with 10% lactose in PBS greatly reduced alterations if done 1 minute after the toxin (Fig. 2D), whereas it had much less effect if done after 5 minutes (Fig. 2E).

Microscopically, there were pathologic findings in all treated eyes 24 hours after application of ricin. Mild inflammation with intraepithelial neutrophils was observed in the tarsal and bulbar conjunctiva when 1 μg was used (results not shown). In a rabbit that received 10 μg, necrotizing inflammation of the nictitating membrane was observed (results not shown). Edema and inflammation of the eyelid stroma usually extended from the conjunctival surface up to the orbicular muscle, with lesser involvement of the cutaneous surface (Fig. 3A). A mild inflammatory reaction was still present in the conjunctiva of rabbits killed 1 week after treatment with 10 μg ricin (Fig. 3B). An acute inflammatory reaction, of increasing severity with higher dosages, was observed in the tarsal and bulbar conjunctiva and in the nictitating membrane of animals 24 hours after receiving 32 μg ricin (Fig. 3C). In most cases, the conjunctival epithelium appeared to be necrotic, with inflammatory membrane formation.

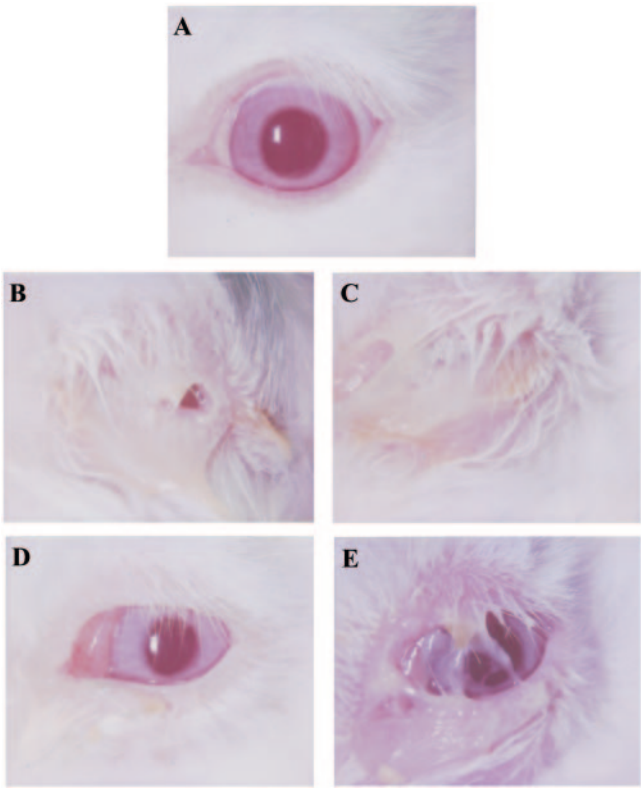

FIGURE 2. (**A**) Eye before treatment. (**B**) Eye 24 hours after 10 μg ricin, showing congestion and intense mucous secretion, with the eye almost not visible. (**C**) Eye treated as in (**B**), washed with PBS 1 minute after application of ricin; the washing had no effect. (**D**) Eye treated as in (**B**), washed with 10% lactose in PBS 1 minute after application of ricin; the condition improved dramatically. (**E**) Eye treated as in (**B**), washed with 10% lactose in PBS 5 minutes after application of ricin, with moderate improvement, clearly less evident than that obtained after rinsing the eye's surface 1 minute after ricin.

The inflammatory infiltrate in the sclera often involved the underlying ciliary body and anterior choroid. Occasional neutrophils in large lachrymal gland ducts were observed in the rabbits treated with 56 or 100 μg ricin.

The sclera showed an acute anterior inflammation in the treated eye of rabbits that received 10, 32, 56, and 100 μg ricin, particularly in the areas adjacent to the conjunctival fornices, with involvement of the extraocular muscles' insertions and orbital fat (Fig. 3D). Again, the severity of the microscopic findings correlated with the ricin dosages. In the rabbit that received the highest dose (100 μg) purulent exudate in the conjunctival fornix was also evident (Fig. 3E).

Mild, acute peripheral stromal keratitis was observed in all treated animals that received >10 μg ricin. The inflammation involved only the central cornea in the three animals that received a 100-μg dose (Figs. 3F, 3G).

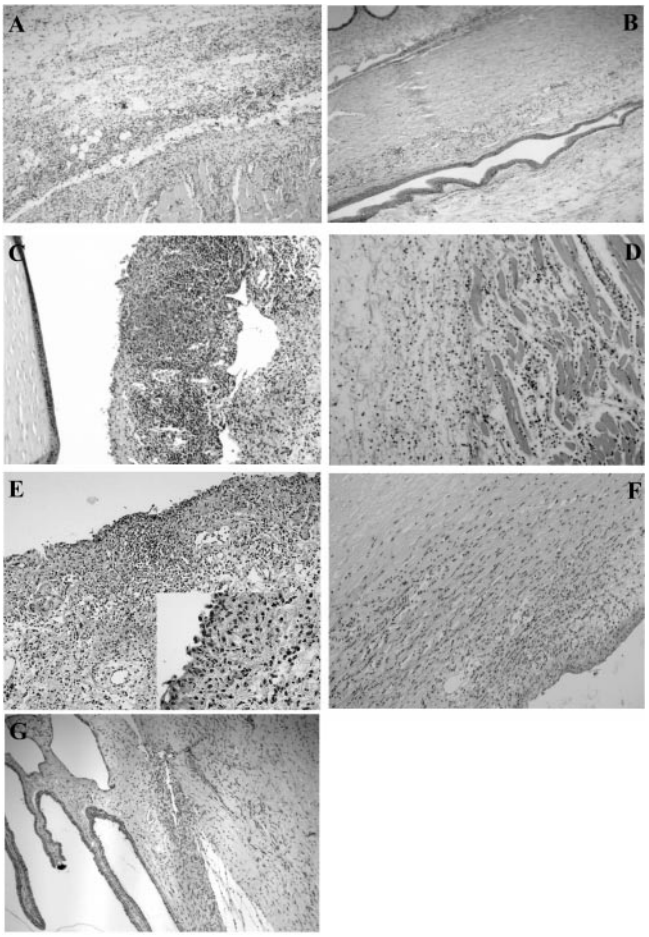

FIGURE 3. (**A**) Eye treated with 10 μg ricin and examined microscopically 24 hours after treatment, showing marked inflammation of the eyelid. (**B**) Eye treated with 10 μg ricin, examined 7 days after treatment, showing moderate inflammation of the limbal conjunctiva and of the third eyelid. (**C**) Eye 24 hours after 32 μg ricin, with the mucosal layer of the third eyelid, on the *right*, showing a dense inflammatory infiltrate of the acute type and foci of tissue necrosis. The well-preserved outer third of the cornea is on the *left*. (**D**) Eye 24 hours after 56 μg ricin, showing a moderately inflamed rectus muscle, with edema and acute inflammation of the overlying orbital soft tissues. (**E**) Eye 24 hours after 100 μg ricin, showing a purulent exudate in the conjunctival fornix. The superficial epithelial layer was largely denuded. Foci of hemorrhage and necrosis were visible in the mucosal stroma. *Inset*: Higher magnification of the inflamed mucosal surface. Hematoxylin-eosin. (**F**, **G**) Same eye as in (**E**), showing (**F**) epithelial and stromal acute keratitis, with the anterior chamber devoid of inflammatory cells, and (**G**) neutrophils from the markedly inflamed anterior orbit invading the iris root and ciliary body, with proximal part of the choroid mildly involved. Magnification: (**B**, **C**, **E**, **F**) ×100; (**E**, *inset*) ×250; (**A**, **G**) ×25.

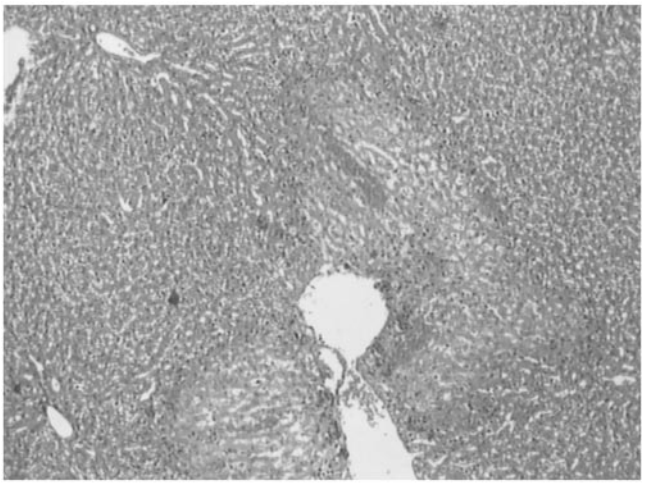

FIGURE 4. In the animals treated with 100 μg ricin, microscopic signs of passive congestion were noted in the liver, with centrilobular sinusoidal dilatation and foci of acinar necrosis in zone 3, surrounded by a mild neutrophilic inflammatory reaction. Magnification, ×25.

The remaining ocular structures and the PBS-treated (control) eyes of rabbits that received <100 μg appeared entirely normal.

In the conjunctiva of rabbits treated with 10 μg ricin followed by washing with lactose and killed after 24 hours, only a mild inflammatory reaction was visible (results not shown).

In the animals that received 100 μg ricin, the histologic examination of liver and kidney revealed foci of hemorrhagic necrosis surrounded by inflammatory cells of the acute type and congestion and severe dilatation of the hepatic centrilobular and midzonal sinusoids, with compression of the hepatic cell plates (Fig. 4). There was no evidence of renal glomerular thrombosis. Marked blood vessel congestion was invariably present in all examined organs.

No gross alterations of internal organs were observed in rabbits that received 32 or 56 μg ricin and were killed after 24 hours or in those that received 10 μg and were killed after 1 week.

DISCUSSION

Ricin applied to rabbit eyes caused severe local damage, affecting mainly the conjunctiva, with lesions that were inflamed to necrotic markedly evident 24 hours after ricin application and visible at doses as low as 10 μg. Mild uveitis and substantial myositis were also present. Corneal involvement consisted mainly of sparse neutrophil infiltration of the peripheral stroma, with no evidence of epithelial damage, whereas a necrotizing inflammatory reaction was observed in the conjunctiva and sclera of the same animals. These results are consistent with the conjunctivitis caused by extracts of castor beans observed by Ehrlich in 1891 (quoted in Ref. 13). The relatively mild involvement of the cornea is noteworthy. This may be caused by the absence of mucin-containing goblet cells in this area, compared with the conjunctiva, and by the sugar specificity of the toxin. Otherwise, it is possible that the toxin present in blood does not reach this tissue, because of the avascularity of the corneal stroma. Eyes appeared macroscopically almost normal 1 week after the application of 10 μg ricin, indicating healing of not too severe lesions.

In rabbits treated with the highest dose (100 μg), lesions were observed also in the contralateral eye. Necrosis and inflammation in liver and kidneys and congestion of the bladder were observed. These changes were unexpected and indicate that molecules as large as ricin can pass into circulation after being absorbed by the mucosae of the eye and/or the nasopharyngeal tract, reached through the lacrymonasal duct. It is possible that the inflammation facilitates the entry of the toxin through the mucosae.[15]

Moreover, the entry into the circulation of even minute amounts of ricin, insufficient to cause histologic damage, could cause the mounting of an immune response. Allergic reactions easily develop in a high percentage of people exposed to allergens present in *Ricinus* seeds.[16] Allergy to ricin has been attributed to a smaller contaminant.[17] However, allergic reactions are caused by various RIPs[18] (Stirpe F, unpublished results, 1975), and ricin enhances the IgE production in response to other allergens.[19]

Rinsing eyes with PBS, even if done immediately after the application of ricin, did not prevent damage to the eye. Instead, rinsing the eye with lactose markedly reduced damage, provided it was done almost immediately after the toxin was applied. These results indicate that ricin, being a lectin specific for sugars with the galactose structure, binds almost immediately to sugar residues on the cell, from which it can be removed by lactose, but not by PBS. The lesser effectiveness of rinsing with lactose after 5 minutes suggests that, at this time, the toxin has already entered the cells, from which it cannot be removed by lactose, and that consequently an irreversible progression toward damage occurs.

From the clinical point of view, no severe visual alterations should be present in the acute phase, when conjunctival lesions only are present. Eye movement impairment most certainly occurs in cases with substantial myositis. Uveitis may cause visual impairment with lesions, which very likely will cause a scarring process. Yet, there are no sufficient elements for establishing whether uveal involvement is due to a local or, in the case of the rabbit that received 100 μg ricin, to a systemic effect of the toxin. Finally, no corneal involvement was detected, except at the highest doses of toxin, perhaps because no vessels are present in this tissue or because of its anatomic characteristics, which provide a higher protection.

On the practical side, present results suggest that in the unfortunate case that the presence of ricin in the air is suspected, not only should inhaling be prevented, but eyes should be protected as well.

Acknowledgments

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