Toxicity of 1, 8, 9-triacetoxyanthracene to the cornea in rabbits

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1, 8, 9-Triacetoxyanthracene, marketed under the trade name Exolan, has been reported to be a useful agent in the treatment of psoriasis (Hellier and Whitefield, 1967). It was introduced to replace the drug Dithranol (anthracene-1, 8, 9-triol), which was previously employed with some success, but had the disadvantage that it caused staining of linen and clothing, and was also associated with burning of the skin. Triacetoxyanthracene does not have these adverse reactions (Hellier and Whitefield, 1967). Recently it was noted that triacetoxyanthracene caused corneal oedema when accidentally introduced into the conjunctival sac of the human eye (Mathalone and Easty, 1967). One of the patients described in this report, using the preparation marketed as a cream suitable for use on the scalp following a shampoo, inadvertently introduced some into both eyes. The other patient, using the paste for a psoriatic eruption on the trunk, suffered a similar corneal lesion, although he did not positively admit to introducing it into his eye. Following these clinical observations, the effect of triacetoxyanthracene on the rabbit cornea was examined in order to elucidate its site of action, and to demonstrate the pathogenesis of the lesion produced.

Method

Different quantities of Exolan were introduced into the conjunctival sac of one eye of each of six rabbits. This treated eye was compared with the contralateral (control) eye into which was instilled an equivalent concentration of the base suspended in water. The dose-levels of triacetoxyanthracene were; 2 per cent. (2 rabbits), 0.66 per cent. (1 rabbit), 0.2 per cent. (1 rabbit), and 0.1 per cent. (2 rabbits). One rabbit, into the eye of which 2 per cent. triacetoxyanthracene had been instilled, was assessed over a period of 10 weeks.

The lesions produced were examined with the slit-lamp microscope and at the same time the corneal thickness was measured using the pachometer developed by Maurice and Giardini (1951). The permeability of the epithelium and endothelium was used as a test of cellular function employing the fluorophotometric apparatus and technique as described by Maurice (1963).

The epithelial permeability was measured by holding an aqueous solution of fluorescein (0.5 per cent.) in contact with the cornea for a period of 1 minute, after which the eye was irrigated with 0.9 per cent. saline. The fluorescein concentrations were measured 2 hours later in the stroma of both the treated and the control eyes and were compared. In the measurement of the endothelial permeability, similar control studies were made as in the epithelial studies. Fluorescein concentrations were measured in the stroma and aqueous at 30-minute intervals over a period of 4 hours. The ratio of stromal to aqueous fluorescein was calculated on each occasion, and from these results a decay curve was constructed. All experiments were performed on eyes which had been treated with triacetoxyanthracene at least 24 hours previously.

The histopathological changes were examined using haematoxylin and eosin, and the histochemical characteristics were studied using Alcian blue, the periodic acid-Schiff reaction, and

colloidal iron staining, as suggested by Ashton (1959). The endothelium was stained in flat preparation using trypan blue, and examined for cellular damage (Stocker, King, Lucas, and Georgiade, 1967).

Results

The effect of one drop of 2 per cent. triacetoxanthracene suspended in an oily emulsion was assessed over a period of 10 weeks. 3 hours after the introduction into the conjunctival sac, there was a diffuse geographical type of corneal staining with fluorescein. The stroma showed no opacity, and there were fine folds in Descemet's membrane, which surrounded islands of normal endothelium. The conjunctiva showed no reaction, and the corneal thickness increased by 15 per cent. 24 hours later, the palpebral fissure was slightly narrowed, and the ciliary vessels were injected. The epithelium was faceted and irregular but did not stain. The superficial part of the stroma was opaque and oedematous. Large dellen were present and these disappeared and reappeared in different parts of the cornea (Fig. 1), being particularly noticeable in the nasal quadrants in the area overhung by the nictitating membrane. After 4 days, the eye was less injected but the ciliary flush was still present. The corneal thickness had increased by 100 per cent. At 6 days, the cornea was grossly oedematous and the superficial half was laminated and opaque (Fig. 2). The corneal oedema remained in this state until the 16th day, at which time the oedema began to clear from the temporal side. During the intermediate period,

FIG. 1 Rabbit cornea 24 hours after treatment with triacetoxanthracene, demonstrating the presence of dellen

FIG. 2 Gross opacification of rabbit cornea 5 days after treatment with triacetoxanthracene

corneal vascularization advanced progressively towards the centre and was both superficial and deep stromal in type. Isolated vessels reached the central area at the end of the first month. About this time, a white soapflake plaque of epithelial hyperplasia appeared near the centre (Fig. 3). With the further passage of time superficial pigment deposits appeared on the nasal side of the cornea, beneath the area covered by the nictitating membrane. At 5 weeks, the area of corneal clearing had progressed nasally, and the plaque of epithelial hyperplasia was still in evidence. Eventually the oedema subsided and the vascularization withdrew to the marginal area as shown in Fig. 3, while the epithelial plaque finally disappeared.

The effect of different concentrations of the toxic agent was examined; 1 drop of 0.2 per cent. triacetoxanthracene produced no change in physical appearance of the cornea or its thickness, while one drop of 0.66 per cent. of the reagent caused an increase in thickness

FIG. 3 Rabbit cornea at 5 weeks, showing epithelial soap-flake hyperplasia with peripheral vascularization. The stromal oedema has resolved.

of 90 per cent. in 5 days, with commensurate changes in the conjunctival and limbal vascularization. When a concentration of 0.1 per cent. triacetoxanthracene was held in the conjunctival basin for 4 minutes, the increase in thickness 2 days later was 80 per cent. In this experiment there was minimal ciliary injection.

PERMEABILITY STUDIES

The measurement of the permeability of the epithelium in a rabbit which had been treated with 0.1 per cent. triacetoxanthracene for 4 minutes, 2 days previously, indicated that it had increased by a factor of 50. The ratio of fluorescein in the stroma and aqueous was found to be 6 : 1 in both the treated and the untreated eyes. The decay curve of fluorescein loss from the stroma paralleled that constructed for the control eye. Thus the permeability of the endothelium was unaffected. Other similar studies on two other rabbits substantiated this finding, that the permeability of the epithelium was affected more than that of the endothelium.

PATHOLOGY

Sections of a cornea treated with a 2 per cent. solution of triacetoxanthracene 6 days previously were stained with haematoxylin and eosin and it was found that the epithelium was thinned down to one or two layers of cells (Fig. 4). There was a slight inflammatory

FIG. 4 Rabbit cornea treated with triacetoxanthracene 6 days previously, demonstrating a normal endothelium below, and an attenuated layer of epithelium above. $\times 70$

reaction at the limbus associated with a cellular exudate of polymorphs and round cells. Using differential staining techniques, there was no indication that either collagen or mucopolysaccharide had been preferentially affected. The stromal changes were those that might be expected in any type of corneal oedema. Using trypan blue in the study of the endothelial viability, the damaged cornea did not stain differently from the normal.

Discussion

The toxic effect of triacetoxanthracene on the cornea is now indisputable, though this is not necessarily permanent, the lesion being one of stromal oedema that does not appear to result in scarring. The reaction can best be described as non-necrotizing, using the classification suggested by Aronson, Yamamoto, Enterline, and Bedford (1967). It is of some importance that there is only a slight inflammatory reaction at the limbus when the toxin is used in dilute concentrations, and the lesion may present clinically as an isolated corneal opacity producing blurred vision with neither subjective nor objective findings suggestive of an inflammatory basis. This was the situation in one of the cases originally reported (Mathalone and Easty, 1967). A recent report on the value of triacetoxanthracene in psoriasis states that one of its side-effects is conjunctivitis (Hellier and Whitefield, 1967). This is clearly understating the situation. The makers do stress the importance of not introducing the drug into the eyes; however, in a product marketed as suitable for use following a shampoo, such an accident would seem inevitable on occasions.

Both the permeability studies and the histopathology show that damage to the epithelium appears to be the main factor in the production of the oedema. Whether this is because the cell layers were reduced in number and the barrier function was consequently reduced, or whether it was the direct result of its toxic effect upon the metabolism of the remaining layers of cells in the epithelium cannot be said, though the former appears the more likely. At the same time, these factors are not mutually exclusive and it may be that they both help to produce stromal oedema.

The endothelium was judged to be healthy on three criteria; normal permeability, normal histopathology, and normal reaction to staining with trypan blue. Although these three criteria suggested that the endothelium was normal, they could not categorically rule out a toxic effect on the inner cell layer.

Corneal vascularization is often induced for experimental purposes by injecting toxic agents into either the anterior chamber or the corneal stroma (Graymore and McCormick, 1968). Agents that produce corneal oedema by external application in low concentration, without inducing stromal damage of a lasting nature in the form of scarring, are uncommon. Triacetoxanthracene through its toxic effect may be of some value as a simple method of inducing experimental corneal oedema and vascularization when required.

Manufacturers of drugs or cosmetics sometimes employ experimental methods based upon a series of photographs showing varying levels of conjunctival injection and corneal oedema, but without the aid of a corneal microscope. It would appear that assessment by the measurement of the corneal thickness and permeability might be a valuable aid in the investigation of these types of toxic effect. Corneal thickness seems to be a particularly sensitive method for determining early corneal changes as shown by the 15 per cent. increase found in an eye that had been treated with 2 per cent. triacetoxanthracene only 3 hours previously and which showed no other significant changes except some irregular staining with fluorescein.

Summary

- (1) The effect of triacetoxanthracene on the rabbit cornea has been investigated, and the drug has been found to be severely toxic, causing marked stromal oedema and epithelial thinning in low dilutions.
- (2) The results of histopathological studies of the corneal sections suggest that the main toxic action is exerted upon the epithelium and not the endothelium. This is substantiated by the permeability experiments.
- (3) It is suggested that measurement of the corneal thickness and permeability is a delicate and useful means of assessing the toxicity of drugs or cosmetics upon the cornea in laboratory animals.
- (4) Topical application of triacetoxanthracene is suggested as a useful agent in the production of experimental corneal oedema and vascularization without the injection of foreign substances into the anterior chamber or corneal stroma.
- (5) The use of triacetoxanthracene after hair shampoos seems to be contraindicated. Scrupulous care should be taken in washing the hands after applying the agent to any part of the body.

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