Clearing media for the eye

DAVID M MAURICE

From the Division of Ophthalmology, Stanford University School of Medicine, Stanford, CA 94305, USA

SUMMARY Non-ionic radiological contrast media have a high refractive index and are not toxic to living cells. They can be used as clearing media for unfixed ocular tissues. Corneal opacities in the rabbit could be made transparent, on a temporary basis, by soaking the stroma in contrast media, and no ill effects on the eye were noted.

It is an established technique to make tissues transparent by impregnating them with a solution of a high refractive index which matches that of the structural proteins and reduces their light scattering. In this way stereoscopic views of their internal structures can be obtained at a moderate magnification. This has required fixing the tissue, dehydrating it in alcohol, and soaking it in an organic solvent of high refractive index. Potts¹ established the optimal index to be 1.55 and recommended a mixture of methyl salicylate and oil of anise as being pleasanter to use than the more customary nitrobenzene. Water-soluble salts containing iodine can also achieve a sufficiently high refractive index, but the very high concentration of ions causes the collagen to shrink and the tissue to become distorted, as in the case of an ionised radiological contrast medium² or of potassium iodide.³

Recently non-ionised radiological contrast media have been introduced which contain a large proportion of iodine and accordingly have a high refractive index in solution. Two such, metrizamide (Amipaque*) and iohexol (Omnipaque*), are alcohols containing three iodine atoms per molecule. The potential of these solutions as clearing agents were examined *in vivo* as well as on isolated ocular tissues. Iohexol is supplied by the manufacturers in a sterile and stable solution at a concentration of 35 g/100 ml, while metrizamide can be made up to the same concentration from lyophilised powder that is readily available. The refractive index of these

solutions is 1.46, and their tonicity was measured to be about twice that of tissue fluids.

Materials and methods

Ocular tissues, either fresh or fixed in formaldehyde and washed, were blotted dry and immersed in a small volume of the solution of contrast medium.

Central corneal leucomata were produced in albino rabbits while under ketamine general anaesthesia supplemented by topical proparacaine. Either about 10 µl of 1 N HCl was injected into the centre of the stroma or a small circle of filter paper soaked in 1 N NaOH was laid on the epithelial surface for a few seconds. In either case there was very little inflammatory response, but a central white scar was produced in the cornea which did not resolve in the succeeding days.

Treatment with the clearing agents was carried out on the animals similarly anaesthetised. If the epithelium had regrown after the chemical injury, it was scraped away from the central area with a scalpel blade. In some eyes the solution was applied in drops about every 30 s to the centre of the cornea, which was directed upward; in others a short plastic cylinder was pressed on to the eye round the limbus and sufficient solution was instilled into it to cover the apex of the cornea. When the treatment was prolonged for an hour, a cup filled with the solution was held over the cornea with a light suction.

Results

IN VITRO

Fixed human and fresh rabbit and beef tissues cleared very completely after about a half hour of soaking. Albino rabbit sclera became virtually invisible to the naked eye. Details of the vascularisation and pigmen-

Correspondence to Dr David M Maurice at the Division of Ophthalmology, S030, Stanford University Medical Center, Stanford, CA 94305, USA

*Amipaque was supplied by Winthrop Laboratories, Sterling drug Co., New York, NY, and Omnipaque was a gift from Sterling-Winthrop Research Institute, Rensselaer, NY.

Fig. 1 Left: Corneal scar created by caustic soda. Right: The same eye after removal of the epithelium and 20 minutes treatment with iohexol. The pupil was dilated to provide photographic contrast.

tation can be observed under a dissecting microscope; the view was similar to that seen after treatment according to the method of Potts. Iohexol seemed slightly superior to metrizamide, but this was difficult to quantitate. The iris similarly cleared to allow satisfactory visualisation of its structure.

IN VIVO

In the eyes in which a large central corneal scar had been produced by acid or alkali no details of the retina could be seen with an indirect ophthalmoscope. Soon after treatment of the cornea with the medium it began to clear, and in about 20 minutes it became sufficiently transparent for full details of the fundus to be observed, as in a normal eye. The anterior segment could equally be examined with the slit-lamp through the cleared area. The stroma was seen to have thinned during the treatment. The scarred cornea remained fairly clear for 10 or 20 minutes after the treatment was stopped and then reverted to its previous state. (Fig. 1.)

Subconjunctival injections of about 50 μ l of solution were made in albino rabbits, and the region of the ciliary body was examined in the slit-lamp to find out whether a better view of this structure and its circulation could be obtained. However, no substantial improvement was seen.

Similarly, in other eyes the aqueous humour was totally evacuated through a needle and then replaced with the clearing medium. This did not make a marked difference in the visibility of the structures and vessels of the iris.

TOXICITY

There was no permanent ill effects to the eye in these

experiments, but a risk of injury to the tissues, particularly the corneal endothelium, is present, and further studies were made with greater exposure to the drugs.

Three normal corneas had their epithelium scraped off over a 6 mm diameter circle and were subjected to iohexol treatment for an hour rather than the 20 minutes needed to return clarity to the opaque cornea. The epithelium regrew without incident, and a few days later the only residual sign was a slight change in the refringence of the anterior stroma visible only in the slit-lamp. In two of the eyes the epithelium of the opposite eye was scraped off over the same area, but no treatment was given. The eyes were stained with fluorescein and observed under the slit-lamp daily thereafter. The epithelium regrew essentially at the same rate on both sides, the control eye being slightly advanced in one case and delayed in the other. The stroma was swollen in the bare area, as was to be expected, and was not substantially different on the two sides, as measured with an ultrasonic pachometer. This small difference does not suggest that any injury to the endothelium had occurred. Two eyes similarly treated for 1 h with metrizamide also recovered without incident and afterwards also showed a slight but rather great change in refringency of the anterior stroma.

In eyes where the aqueous humour was totally replaced with the clearing medium, two in each case, no signs of injury to the tissues and little inflammation was noted in spite of the high osmotic pressure of the solution and the absence of glucose and essential electrolytes. One day later the cornea had returned to its normal thickness, and two days later the needle track was the only sign of the intervention that could

be noted with the slit-lamp or the naked eye, both the cornea and lens being indistinguishable from the control eye. Further testing did not seem warranted, since the threshold to injury for the human eye could be different from that of the rabbit.

The undiluted contrast media were also tested for their toxicity to primary or first-passage monolayers of tissue cultured rabbit corneal endothelium and keratocytes. The supernatant was sucked off and the cells were incubated under a thin layer of the contrast medium for various times before being covered again with tissue culture medium. It was found that both types of cells could withstand exposure to the contrast medium for up to 30 minutes without any morphological changes being noted at any time. After this they began to round up, and longer exposures led to detachment and apparent death for some of the cells.

Discussion

Non-ionic radiological contrast media are preferable to oils as a clearing medium for excised tissue samples. They have the advantage of simplicity and speed, and the treated tissue will remain in a more normal, possible viable, condition.

In the clinic it is possible that they will allow observation of the anterior chamber and the fundus in patients with scarred or oedematous corneas. It should also make it possible for these patients to estimate the benefit they might achieve from a

corneal transplant. The barely detectable changes in the stroma would not be of importance in these cases, and from the few animal experiments it does not seem probable that injury to the epithelium or endothelium will occur; this needs to be tested in patients.

These media might have advantages over glycerol, which needs an intact epithelium in order to withdraw water osmotically from oedematous corneas and which can cause injury to the endothelial cells.⁴ On the other hand their administration requires scraping away the epithelium from enough of the corneal surface to allow a view into or out of the eye, and this may be unacceptable as a procedure in many cases.

Ms Tejinder Singh and Ms Frances Monroe provided invaluable assistance.

The work was supported by National Institute of Health grant EY 00431.

References

- 1 Potts AM. Anatomic methods for study of the bulbus oculi. *Am J Ophthalmol* 1968; **65**: 155–63.
- 2 Maurice DM. The cornea and sclera. In: Davson H, ed. *The eye* 2nd ed. London: Academic Press, 1969: 1: 525.
- 3 Cress J, Maurice DM. An attempt at chemical treatment of myopia. *Arch Ophthalmol* 1971; **86**: 692–3.
- 4 Goldberg MH, Koffler BH, Lemp MA, Rao NA. The effects of topically applied glycerin on the human corneal endothelium. *Cornea* 1982; **1**: 39–44.

Accepted for publication 31 July 1986.