ORIGINAL ARTICLES — Laboratory science

Mitomycin against posterior capsular opacification: an experimental study in rabbits

Christine M Haus, Albert L Galand

Abstract

Aims/background—Posterior capsular opacification is the most common postoperative complication of extracapsular cataract surgery. The purpose of this study was to attempt to inhibit this secondary cataract formation by using mitomycin (an antimitotic drug).

Methods—A solution containing mitomycin was used to perform hydrodissection (with a 5 minute pause) during extracapsular lens extraction in rabbits. This way of administration was chosen to reduce as much as possible drug diffusion into the anterior chamber. Heparin was added to the irrigating solution to avoid fibrin formation. Its ability to prevent posterior cataract opacification was also evaluated at the end of the study. The animals were sacrificed 4 or 6 months after surgery. Grading concerning two aspects of secondary cataract (proliferation and fibrosis) was obtained on gross examination. Histological analysis was subsequently performed.

Results—This study demonstrated that mitomycin has a significant inhibitory effect on secondary cataract formation (proliferation as well as fibrosis) in rabbits whereas heparin does not seem to have the same effectiveness.

Conclusion—This work is a preliminary study concerning the use of mitomycin for prevention of posterior capsular opacification. It has proved its effectiveness in rabbits but more in depth studies are still necessary before its application in humans.

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(2) lens epithelial cells may undergo metaplasia to myofibroblasts; these cells have contractile properties similar to smooth muscle cells and their contraction produces numerous tiny wrinkles in the posterior capsule resulting in visual distortion.

In both cases, the opacification has its origin in the residual lens epithelial cells which are in the 'germinative area' at the capsular bag equator. We may therefore presume that a good way to prevent development of opacities of the posterior capsule is to act at the germinative area level to destroy the epithelial cells during surgery.

In our study, we attempt to prevent secondary cataract formation by means of endocapsular application of mitomycin during extracapsular extraction in rabbits.

Material and methods

The lenses of 26 eyes of 22 rabbits underwent extracapsular extraction all by the same operator. White young rabbits (Blanc de Termonde) weighing 2.5-3 kg and free of eye disease (that is, with clear lenses) were used in this study.

Adequate pupillary dilatation was performed by repeated instillations of phenylephrine hydrochloride (Phényléphrine, Unipede) and cyclopentolate hydrochloride (Cyclopentol, Asta Medica). The animals were anaesthetised with an intramuscular injection of xylazine hydrochloride 5 mg/kg body weight (Rompun, Bayer) and ketamine hydrochloride 50 mg/kg body weight (Ketalar, Parke-Davis). Topical application of oxybuprocaine hydrochloride (Novesine, Bournonville Pharma) was used for local anaesthesia. The ocular area was disinfected and a wire lid speculum was inserted to retract the lids.

A corneal incision was made by a 3.2 mm calibrated blade (Alcon). A volume of 0.5 ml of solution I (balanced salt solution (BSS), with or without heparin (Heparine Leo, Leo Pharmaceutical Products, Ballerup, Denmark) according to the group) was injected in the aqueous humour and, after anterior chamber reformation with sodium hyaluronate (Healon, Pharmacia), a continuous curvilinear capsulotomy of about 5 mm in diameter was performed using a capsular forceps. The anterior chamber was again filled with Healon which forms a protective layer under the endothelium.

Department of
Ophthalmology,
University of Liege,
Belgium
C M Haus
A L Galand

Correspondence to:
Dr C Haus, Department of
Ophthalmology, CHU
Sart-Tilman, B-4000 Liege,
Belgium.

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Extracapsular cataract surgery preserving the posterior capsule retains an avascular membrane between the vitreous cavity and the anterior chamber. The most common postoperative complication of this surgery is the opacification of the posterior capsule. The opacification occurs in two ways¹⁻³:

(1) residual lens epithelial cells may proliferate and migrate onto the posterior capsule (without forming tight junctions with it) producing new lens fibres which create frank opacities;

The hydrodissection was then performed very cautiously, exercising particular care when injecting 0.15 ml of solution II (BSS, with or without mitomycin (Mitomycin C Kyowa, Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan)) around the cortex and nucleus so that this solution did not cross through the anterior capsulotomy. Five minutes later, the hydrodissection solution and some cortex were sucked up. The nucleus and cortex of the lens were then loosened; the incision was enlarged with Castroviejo scissors and the nucleus and adherent cortex were expressed from the eye. Any residual lens cortical material was then removed by irrigation and aspiration (I/A).

Solution III (balanced salt solution, with or without heparin) was used for irrigation. The different solutions are numbered I, II, or III according to the time of their use (solution I is injected in the anterior chamber before making the capsulorhexis, solution II is the hydrodissection solution, and solution III is the irrigant used during lens extraction) but their composition varies in the four different eye groups—A, B, C, and D.

In group A (control), solutions I, II, and III were BSS with bicarbonate dextrose and glutathione (BSS plus, Alcon). In group B (mitomycin), solutions I and III were BSS plus but solution II was a mitomycin solution 0.02% (pH 7.5 and osmolality 445 mosm/kg). This solution represents a total administered dose of 0.03 mg of mitomycin. In group C (heparin), solutions I and III were BSS plus with heparin (respectively 10 U heparin/ml and 5 U heparin/ml), in order to try to prevent fibrin formation. Solution II was BSS plus alone. In group D (mitomycin plus heparin), solutions I and III contained heparin as in group C. Solution II contained mitomycin as in group B.

The operator was unaware of the type of solution employed.

For all groups at the completion of the procedure, the corneal incision was closed with interrupted nylon 10-0 sutures. Postoperatively, all surgical eyes were treated topically with gentamicin (Ophtagram, Chauvin) daily for 7 days.

Four or 6 months after extracapsular lens extraction, the animals received an intravenous overdose of pentobarbitone (Nembutal, Abbot). The eyes were enucleated and then dissected for removal of the cornea and the iris, leaving the secondary cataract and a scleral rim intact.

The grading was obtained on gross examination. The posterior capsular opacification was studied for evidence of proliferation and fibrosis.

The density of proliferation was scored from 0 to ++++ taking into account the opacified surface area, the region (peripheral or central), and the thickness of the proliferation: 0 signifies a clear capsule; + relates to a low density peripheral involvement of a quarter of the total capsule area; ++ represents a medium density peripheral involvement of half of the total capsule area; +++, a high density total peripheral involvement of the capsule area; and +++++, a high density peripheral and central

Figure 1 Aspect of fibrosis 4 months after extracapsular extraction in rabbits.

involvement of the capsule area. The fibrosis (Fig 1) was evaluated from 0 to +++ according to the importance of opacification, the presence of folds, and their localisation.

This grading system is subjective but was carried out by only one ophthalmologist unaware of which group the eyes belonged to and who used the same scoring system for all eyes observed.

After grading, the specimens were fixed with 10% neutral buffered formalin and stained with haematoxylin and eosin for histological examination.

During the study, three rabbits died and so were excluded from the analysis since their follow up could not be completed.

All experimental animals were treated in accordance with the legislation of the Belgian French Community on the Use of Animals in Research.

Results

In the initial postoperative period, some fibrin was present in most eyes. This anterior chamber inflammation spontaneously resolved by the end of the second week. The corneas stayed relatively clear. There was no objective measure of the intraocular pressure, but we did not observe any sign of ocular hypertension.

Table 1 presents the grading obtained for each protocol (group A, B, C, or D) together with the postoperative survival period (all eyes were studied 4 months after surgery except those indicated as 6 months in the table).

Table 1 Evaluation of the inhibitory activity of mitomycin and heparin on proliferation (P) or fibrosis (F) after extracapsular extraction in eyes of rabbits

	A Control (n=7)	B Mitomycin (n=7)	C Heparin (n=5)	D Mitomycin/ heparin (n=4)
P	+++	+	+++	0
F	+	+	+	+
P	++++	+	++	+
F	++	0	+++	+
P	+++	+	++++	+
F	+	+	++	0
P	+++	+	++++	++
F	++	0	++	+
P	++++	0	++++	
F	++	++	+	
P	++++	0		
F	++	0		
P	++	+		
F	++	+		

*Studied 6 months after surgery.

Figure 2 Evaluation of proliferation with or without mitomycin after extracapsular extraction in rabbits.

Figure 3 Evaluation of fibrosis with or without mitomycin after extracapsular extraction in rabbits.

Proliferation 0 was only found in protocol B or D (mitomycin treated eyes). No high grade (+++) fibrosis was observed. Comparing the first and the second columns, proliferation is always higher in protocol A (control) than in B (mitomycin) while fibrosis is higher or identical but never lower. Protocols B (mitomycin) and D (mitomycin and heparin) have the same grading for proliferation as well as for fibrosis, which suggest that heparin does not have any additional effect.

The heparin treated eyes did not present unexpected bleeding during or after surgery and, in general, postoperative inflammation was less severe. But when comparing results from the first and the third columns, heparin does not seem to reduce either proliferation or fibrosis.

Figure 2 illustrates only the 'proliferation' aspect of the secondary cataract. It presents for each level of grading the number of eyes with (protocols B and D together) or without mitomycin (protocols A and C together).

Most eyes without mitomycin appear to have a high level of proliferation whereas most eyes with mitomycin do not. χ^2 analysis with regrouping (little or no proliferation (0 and +) compared with higher grades of proliferation (++ , +++ , and ++++)) shows that proliferation is significantly reduced with the use of mitomycin ($p < 0.0001$).

Figure 3 concerns fibrosis. This seems to be the same as for proliferation but the difference between eyes with or without mitomycin is not as clearly marked as in Figure 2. Fibrosis is significantly decreased ($p < 0.005$ with χ^2 analysis with the same regrouping as for proliferation).

Histological examination of the proliferation shows in all groups some retained lens epithelial cells and cortical material in the Soemmering's ring, and some cells which have migrated along the posterior capsule which is normally free of cells. This is similar to what is observed in humans.⁴

Discussion

Previous studies have demonstrated that lens regeneration after extracapsular extraction in rabbits occurs spontaneously and follows the stages seen in the normal embryonic development of the lens. If lens capsular bag integrity is restored this will lead to a structure resembling a new lens.⁵ But if there is a capsulectomy, the anterior rim will adhere to the posterior capsule so that lens regrowth will first be contained between the two capsules

Figure 4 Soemmering's ring 4 months after extracapsular extraction in rabbits.

Figure 5 Aspect of proliferation (++++) 4 months after extracapsular extraction in rabbits.

giving the newly formed lens the doughnut shape of the Soemmering's ring (Fig 4).⁶ With time, irregularity in fibre alignment appears and the lens becomes progressively opaque.⁷ Proliferation continues towards the central area but with different characteristics from those seen in humans (Fig 5).

Young albino rabbits are very useful for testing the efficiency of a technique that would prevent posterior capsular opacification because they form florid secondary lens material within a short time after extracapsular lens extraction (human lens epithelial cells produce significant secondary cataracts in months rather than weeks). But the rabbit model is an extreme model. It is not only distinguished from the human eye by the large amount of de novo synthesis of crystalline proteins by epithelial cells but also by the severe postsurgical inflammation (which may play a role in secondary cataract).

To reduce this postoperative inflammatory reaction (related to fibrin exudation in the anterior chamber) heparin is usually used in

rabbits because of its well known inhibitory effect on fibrin formation from fibrinogen in the coagulation cascade.

With reference to the study of Knorr *et al* which showed the dose dependent inhibition of heparin on cultivated bovine lens epithelial cell proliferation⁸ we have also evaluated the capacity of heparin to inhibit secondary cataract formation.

Zaturinsky *et al*⁹ found that heparin supplementation in the irrigating solution (100 IU/ml) by itself reduced posterior capsular opacification at a late postoperative stage. This is probably related to heparin's anti-inflammatory properties, pointing to the possible role of inflammation in secondary cataract formation, at least in rabbits.

Use of heparin coated intraocular lenses also produces a long term reduction of cell deposits on the intraocular lens surface resulting from decreased inflammation. However, in Spangberg and colleagues' study,¹⁰ posterior capsular fibrosis was not less in eyes implanted with heparin surface modified intraocular lenses.

In practice, heparin can be added to the irrigating solution^{7,9,11} or injected into the anterior chamber before the capsulectomy.¹² In our study we have combined these two methods of administration but our results do not really confirm the inhibitory effect of heparin on secondary cataract formation. This lack of inhibitory effect compared with previous studies may be related to the use of different concentrations of heparin. In fact, we decided that the combined administration allowed us to reduce both concentrations; but it may be that the concentrations selected were then below the inhibitory threshold.

We also presumed that the role of the inflammatory response (which is decreased with heparin) in secondary cataract formation exists but was not, in our study, the decisive factor.

Mitomycin is an antineoplastic antibiotic produced by *Streptomyces caespitosus*. It acts as an alkylating agent after in vivo activation by enzymatic reduction with the production of free radicals. Mitomycin is thus toxic because it induces lipid peroxidation through the intermediate of the oxygen radicals.¹³⁻¹⁶ Therefore, it is a cell cycle non-specific agent, although it is most active in the late G1 (gap) and early S (synthesis) phases of the cell cycle.¹³⁻¹⁶

A drug that has only antimetabolic properties inhibits DNA synthesis, results in S phase cell death, and allows cells in other phases to survive and proliferate. So, for this drug, unlike mitomycin, multiple applications will be necessary for it to be effective. While mitomycin's greater cytotoxicity is attractive for use in the prevention of posterior capsular opacification, it poses real problems where the safety of the other ocular structures concerned. Derick *et al* concluded that mitomycin C is toxic to intraocular tissues when directly administered intracamerally in animals.¹⁷ Yet Hayasaka *et al* found the postoperative instillation of 0.02% mitomycin C, twice a day for 5 days, to be effective and safe—no systemic toxicity and few local complications—in the treatment of primary pterygium.¹⁸

In their study, Kitazawa *et al* suggest that the optimum dose of mitomycin for initial trabeculectomy may be between 0.02 and 0.2 mg (the total dose administered in our study was 0.03 mg of mitomycin).¹⁹

Because of the high regeneration rate of the corneal endothelium in rabbits, we have not investigated endothelial damage. In humans, the endothelium does not regenerate, so we might suppose that it will be protected against an antimetabolic action but we cannot exclude damage related to toxic properties.

With our technique of administration (during hydrodissection) we hope to have as minimal diffusion of the product in the anterior chamber as possible and to have effective endothelial protection with Healon. Bovine endothelial cell cultures have shown that, after an exposure period of 5 minutes, only a mitomycin concentration of 3.0 mg/ml caused in vitro endothelial damage.²⁰ This concentration is much higher than the expected intracameral concentration of mitomycin after injection of our solution II.

During our experiment all the corneas stayed relatively clear. Immunolocalisation with antimitomycin antibodies or radiodetection with a radioisotope could be useful in determining where mitomycin is concentrated as well as its potential resorption or eventual accumulation in ocular tissues. Electrophysiological tests could also be performed to determine any effects on the retina, although 2 µg of mitomycin have already been safely injected into the vitreous cavity in rabbits.²¹

Our study demonstrates that mitomycin can effectively inhibit posterior capsular opacification after cataract surgery in rabbits. The method of drug administration during hydrodissection is thought to reduce diffusion in the anterior chamber. Healon is used to protect the endothelium. More in depth studies are still necessary on the toxicity and diffusion of the drug.

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