Estimation of sodium fusidate* levels in human serum, aqueous humour, and vitreous body

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The blood–aqueous barrier prevents, in the healthy eye, the passage of large molecules like those of ampicillin from reaching satisfactory therapeutic levels in the anterior chamber (Kurose, Levy, and Leopold, 1950). Systemic antibiotics are prescribed for patients suffering from intraocular sepsis in the belief that the antibiotic penetrates into the inflamed eye secreting a plasmoid aqueous with or without cellular elements. However, many such cases do not progress satisfactorily and it becomes incumbent on the ophthalmologist to demonstrate in the human aqueous effective therapeutic levels of antibiotics preferably with marked anti-staphylococcal activity.

The first two purposes of this study were (1) to devise a simple, safe technique for the removal of aqueous in human subjects, and (2) to estimate the level of sodium fusidate in aqueous and serum simultaneously. Sodium fusidate was chosen because of its marked action against *Staphylococcus pyogenes* both *in vitro* (Godtfredsen, Roholt, and Tybring, 1962; Barber and Waterworth, 1962; Hilson, 1962; McDonald, 1965; Harvey, Sih, and Knight, 1965), and *in vivo* (Scowen and Garrod, 1962; Taylor and Bloor, 1962; Cusack, 1962; Newman, Bhat, Hackney, Robinson, and Stewart, 1962; Thibault, 1962; Crosbie, 1963; Porter and Wilson, 1963; Jensen and Lassen, 1964; Healy, 1966; Saggars, Harwood, and Day, 1968).

Patients who have had several attacks of uveitis may come to surgery for cataract extraction. They may be regarded as in need of antibiotic cover.

The third purpose of this study (3) was to demonstrate the passage of sodium fusidate into the aqueous of patients who had suffered several attacks of iridocyclitis.

Methods

PHASE I

The nature of the study was explained to each of eighteen patients about to undergo routine cataract extraction under local anaesthesia. They were asked to take 500 mg. sodium fusidate three times a day for 3 days (6 patients), for 2 days (6 patients), or for 1 day (6 patients). The final dose of sodium fusidate was given 12 hours before surgery (Table I, opposite).

10 ml. blood for serum sodium fusidate estimation were withdrawn while the retrobulbar anaesthetic was taking effect. The eye was grasped at the insertion of the medial rectus. The tip of a

Table I Serum and aqueous levels of sodium fusidate in patients prepared for cataract extraction

Patient no.	Age (yrs)	Sex	Duration of fusidate therapy (days)	Sodium fusidate levels ($\mu\text{g./ml.}$)		Comments
				Serum	Aqueous	
1	74	M	3	52	1.28	
2	74	M	3	72	1.28	
3	68	M	3	58	1.2	
4	58	F	3	64	1.2	
5	65	M	3	70	1.28	
6	65	M	3	3.2	< 0.16	Patient had not taken tablets as directed
7	62	F	2	64	0.8	
8	60	M	2	64	1.2	
9	58	M	2	40	1.2	
10	70	M	2	18	1.2	
11	58	F	2	64	1.2	
12	62	F	2	64	—	Aqueous sample inadequate
13	57	F	1	28	0.48	
14	64	F	1	36	0.84	
15	60	F	1	16	0.22	
16	70	M	1	32	0.24	
17	80	M	1	4	0.08	
18	74	F	1	23	0.10	

disposable needle (Yale 25 G 5/8 No. 18) connected to a 2 ml. disposable syringe containing 0.5 ml. air was inserted 0.25 mm. from the limbus through the cornea into the anterior chamber. In the case of a patient undergoing right cataract extraction, the needle was inserted at the "9 o'clock" position, and "3 o'clock" was the position for a left cataract extraction. The syringe was held horizontally throughout this procedure. At no time was any difficulty experienced in inserting the tip of the needle. The surgeon's assistant slowly withdrew two or three drops of aqueous and replaced the deficit with air from the syringe. There was no need to detach the syringe or withdraw the needle until the procedure was completed.

Routine cataract surgery was then performed. No difficulty in completing the operation was experienced in any case.

PHASE 2

Two patients with diabetes mellitus, recurrent iridocyclitis, and cataracts were given 500 mg. sodium fusidate three times a day for 3 days, and the cataracts were then removed.

Finally, in order to reduce the formation of aqueous and so reduce the intraocular pressure, carbonic anhydrase inhibitors may be prescribed in patients with intraocular sepsis complicated by glaucoma. The effect of this reduction of flow on the level of sodium fusidate in the aqueous has been investigated (4).

PHASE 3

Eight patients with high myopia were given Diamox 500 mg., and then 250 mg. 3-hrly for 24 hours before operation. 500 mg. Diamox was given intramuscularly on the morning of the operation. This is the standard procedure employed by one of us (J.W.) in such cases. Three of these patients received sodium fusidate 500 mg. three times a day for 3 days, two for 2 days, and three for one day (Table II, overleaf).

Table II Serum and aqueous levels of sodium fusidate in cataract patients treated with sodium fusidate and Diamox

Patient no.	Age (yrs)	Sex	Duration of fusidate therapy (days)	Sodium fusidate levels ($\mu\text{g./ml.}$)	
				Serum	Aqueous
1	70	M	3	70	1.34
2	72	F	3	96	2.56
3	68	F	3	68	1.32
4	59	F	2	40	1.2
5	60	M	2	28	0.84
6	60	M	1	8	0.16
7	61	F	1	24	0.42
8	63	F	1	4	0.03

PHASE 4

During a 6-month period three patients presented with blind painful eyes (Table III).

The first two received 500 mg. sodium fusidate four times a day for 4 days; 12 hours after the last dose the eye was enucleated, and aqueous was removed through the centre of the cornea, and vitreous *via* a small stab wound in the sclera.

The third patient received the same amount of sodium fusidate as the other patients, but the eye was not removed until 4 days after the last dose of Fucidin. Serum, aqueous, and vitreous samples were taken on the day of the operation.

Table III Levels of sodium fusidate in serum, aqueous, and vitreous

Patient no.	Age (yrs)	Sex	Duration of fusidate therapy (days)	Sodium fusidate levels ($\mu\text{g./ml.}$)		
				Serum	Aqueous	Vitreous
1	58*	F	3	84	12.8	28.8
2	60	M	3	56	> 0.64	> 0.64
3	56†	F	3	0.9	0.1	0.32

* Aqueous and vitreous levels excessively high, probably due to increase in protein content associated with prolonged inflammation

† Last dose of Fucidin 4 days before enucleation.

Laboratory technique

Sodium fusidate was assayed by an agar plate diffusion technique using *Corynebacterium xerosis* as test organism. The method was that used by Leo Laboratories Ltd.

Two strains of the test organism, Leo strains FF and FF(M), were sodium fusidate sensitive; strain FF was used constantly. Another strain, FF (ZN6), was sodium fusidate resistant, and was used to detect the presence of other inhibiting agents, either in the material under test, or in the pooled serum used as diluent in the serum and vitreous assays (the assay medium was employed also as a maintenance medium in the form of slopes).

The phosphate buffer used to prepare the standard dilution was adjusted to pH 6.3 since this increased the sensitivity of the assay.

AQUEOUS ASSAY

Holes 6 mm. in diameter were made in the agar and 1 drop of standard or test dilution was placed in each. The standards for this assay were 0.32, 0.16, 0.08, 0.04, and 0.02 $\mu\text{g/ml.}$, measurable

inhibition was constantly observed with 0.04 µg/ml. Aqueous humour was usually diluted 1 in 4 with buffer (1 in 8 if quantity permitted).

Zone diameters of inhibition were measured, and the results transferred to a simple graph of concentration (µg./ml.) against zone diameter of inhibition (mm.), and the aqueous concentration was thus measured. Since an initial dilution of aqueous humour was generally made, the minimum detectable amount of antibiotic in aqueous was usually 0.16 µg./ml.

SERUM AND VITREOUS ASSAY

Standard solutions (6.4, 3.2, 1.6, 0.8, and 0.4 µg./ml.) of sodium fusidate were prepared in pooled human serum. Aliquots were then diluted with three volumes of phosphate buffer. The test serum was usually diluted 1 in 10, and 1 in 20 with pooled serum, and then aliquots were further diluted with three volumes of buffer.

Results

The results of Phase 1 are shown in Table I. As expected, the serum levels of sodium fusidate rose steadily as patients received 1, 2, or 3 days of the antibiotic (1 day mean 23.3 µg./ml., 2 days mean 52.3 µg./ml., 3 days 63.2 µg./ml.). The aqueous levels behaved a little differently in that after only 48 hours adequate therapeutic amounts of sodium fusidate had penetrated into the aqueous (1 day mean 0.33 µg./ml., 2 days mean 1.12 µg./ml., 3 days mean 1.25 µg./ml.).

There was no impediment to the passage of sodium fusidate into the aqueous in the two diabetic patients who had had recurrent bouts of iridocyclitis. Serum levels were 70 and 58 µg./ml. and the aqueous levels 1.32 and 1.2 µg./ml. respectively.

To our surprise, the carbonic anhydrase inhibitor, Diamox, did not reduce the level of aqueous sodium fusidate (Table II). Indeed, some of the highest aqueous levels were obtained in this group of patients, *e.g.* 2.56 and 1.34 µg./ml. However, the overall levels were not significantly higher than in the group of patients who did not receive Diamox.

Sodium fusidate was detected in the vitreous of three patients (Table III). In two cases the vitreous level was between two and three times as high as in the aqueous, and a fairly high vitreous level of sodium fusidate was still present 4 days after the last dose of the drug in one patient.

Discussion

48 hours' treatment with 500 mg. sodium fusidate three times a day produced adequate aqueous levels. It is suggested that the clinician might look for beneficial results from sodium fusidate therapy after 2 days, and that 2 days of therapy should give adequate prophylactic cover.

Since Diamox did not inhibit the penetration of the antibiotic into the aqueous humour, it would appear that sodium fusidate diffuses through the blood-aqueous barrier, requiring no active transportation across the ciliary body epithelium.

Only two patients with a history of repeated attacks of iridocyclitis were included in this survey. In neither did the antibiotic fail to penetrate into the aqueous. However, a larger series should be studied before any conclusions are drawn.

The vitreous levels of sodium fusidate were two to three times as high as the aqueous levels in two patients; 0.32 µg./ml. was found in one patient's vitreous 4 days after the drug had been discontinued. In addition to the same proteins as are found in the aqueous, the vitreous contains vitreïn and mucoid (Adler, 1959). The vitreous, therefore, has a higher

protein-binding capacity than the aqueous though not as high as that of serum. This, in all probability, is the reason why sodium fusidate levels are higher in the vitreous than in the aqueous.

Summary

Sodium fusidate (Fucidin, Leo Laboratories) was demonstrated in the aqueous humour of 25 human patients about to undergo cataract extraction and in three patients requiring enucleation. Adequate therapeutic levels were detected in all the patients who were given either 2 or 3 days' therapy. Neither previous iridocyclitis nor carbonic anhydrase inhibition prevented the passage of the antibiotic into the aqueous. The sodium fusidate had penetrated into the vitreous body of the eyes that had to be enucleated and because of the higher protein-binding capacity of the vitreous the levels of antibiotic were higher there than in the aqueous humour.

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