AQUEOUS KINETICS OF SISOMICIN SULPHATE

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SUMMARY

Sisomicin sulphate is a new-generation aminoglycoside with a broad spectrum of antimicrobial activity that includes *Pseudomonas aeruginosa*. It is superior to gentamicin against indole-negative *Proteus* and some resistant strains of *Pseudomonas*. The ocular pharmacokinetics of sisomicin have not been explored. We used the agar diffusion technique of microbial assay to determine the aqueous penetration and bioavailability of a subconjunctivally placed standard dose of 20 mg/0.4 ml of sisomicin sulphate in 20 human volunteers undergoing elective cataract surgery. A peak concentration of 16.4 mg/l was found in the aqueous humour 78 minutes after injection, which is 65 times the minimum inhibitory concentration for *Pseudomonas*. The antibiotic was bioavailable up to 1203 minutes after injection in a concentration of 0.9 mg/l, which easily covers the minimum inhibitory concentration of *Staphylococcus aureus* and *Pseudomonas*. The antibiotic disappears from the aqueous humour at the 1434 minute interval (approximately 24 hours). The elimination half-life ($t_{1/2}$) of sisomicin was determined to be 5.16 hours ($K = 0.134/\text{hour}$) and the aqueous clearance was 2.87 $\mu\text{l}/\text{min}$.

Sisomicin sulphate is a new aminoglycoside elaborated by *Micromonospora inyoensis*. It acts by inhibiting normal protein synthesis in susceptible organisms. Its chemical structure and pharmacodynamics closely resemble those of gentamicin.¹⁻³ However, the bacterial uptake of the antibiotic is consistently greater and more rapid than that of its sister drug gentamicin.⁴ *In vitro* studies have shown that the drug has a potent bactericidal activity against a broad spectrum of microbes including staphylococci (penicillin- and methicillin-resistant strains) and *Pseudomonas aeruginosa*.⁵ It has been found to be superior to tobramycin against *Serratia* and indole-positive *Proteus*, and also to gentamicin against indole-negative *Proteus* and some resistant strains of *Pseudomonas*.⁶⁻⁸ In sight-threatening eye infections caused by resistant organisms, in which prognosis is poor,^{9,10} sisomicin could be of therapeutic importance. Although sisomicin has a distinctive edge

over other aminoglycosides in some respects,⁵⁻⁸ its pharmacokinetic behaviour in the eye remains unexplored. The present paper aims at determining the aqueous penetration of sisomicin following a standard dose of 20 mg/0.4 ml injected subconjunctivally in human eyes.

MATERIALS AND METHODS

Twenty patients with normal anterior segments, undergoing elective cataract surgery, were enrolled in the study after informed consent was obtained. Any topical, oral or parenteral antibiotics/chemotherapeutics being given were withdrawn 24 hours before surgery. Each patient received 20 mg sisomicin sulphate (Ensamycin injection, Fulford, India) in 0.4 ml solution as a subconjunctival injection in the inferotemporal quadrant, at varying intervals before cataract surgery.

The subconjunctival dose chosen in the present study is equivalent to that of gentamicin, since the pharmacokinetic features of both drugs are similar after parenteral administration.¹⁻³ At the time of surgery, at least 0.1 ml of the aqueous humour was aspirated transcorneally through a 25-gauge disposable needle mounted on a tuberculin syringe. The aqueous samples were transferred to sterile polypropylene bottles and stored at -20°C in a deep freeze unit and later assayed by the agar diffusion technique¹¹ using *Bacillus subtilis* NCIM 2061 strain as the assay organism.

The elimination half-life ($t_{1/2}$) of sisomicin was estimated by the best straight-line fit by eye to the log concentration (aqueous) versus time data obtained in this study. The elimination constant, K , which is the rate constant for elimination of the drug from the aqueous, was determined by the equation:¹²

$$K = \frac{0.693}{t_{1/2}}$$

The clearance of sisomicin from aqueous humour was calculated from the equation:¹³

$$\text{Clearance} = \frac{FX_0}{\int_0^{\infty} Cdt}$$

where F is the fraction of the administered dose X_0 that is

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absorbed following subconjunctival injection (peak aqueous concentration in μg), $\int_0^\infty C dt$ is the area under the aqueous concentration versus time curve from time zero to infinity (calculated by the trapezoidal rule) and K is the elimination rate constant.

RESULTS

An attempt was made to determine the time course of sisomicin accumulation in the aqueous humour by varying the time at which the samples were obtained, after a single subconjunctival injection of the drug was administered. Sisomicin could be assayed in the aqueous humour in a concentration of 1.3 mg/l as early as 18 minutes after subconjunctival delivery (Table I and Fig. 1). A peak aqueous concentration of 16.4 mg/l was assayed at 78 minutes (approximately 1.15 hours) after injection. At peak level, the antibiotic concentration was 65 times the minimum inhibitory concentration against *Pseudomonas* (Table II). Thereafter, the aqueous concentration of the drug decayed until none was recoverable from the aqueous after 1434 minutes (approximately 24 hours). It was observed during the study that after adequate topical anaesthesia (xylocaine), a subconjunctival injection of sisomicin produced mild discomfort in some patients but no pain, conjunctival congestion or chemosis, which shows that the antibiotic is well tolerated by the eye.

The aqueous concentration versus time curve after subconjunctival injection of sisomicin can be described by a one-compartment model with first-order input and output, as in the case of orally or intramuscularly administered drugs. In such a model the $t_{1/2}$, i.e. time taken for aqueous concentration to be decreased by 50%, stands at 5.16 hours ($K = 0.134/\text{hour}$). The clearance of sisomicin from the aqueous humour is calculated to be 2.87 $\mu\text{l}/\text{min}$. This is the rate at which the aqueous becomes freed of sisomicin after peak level is attained.

DISCUSSION

There are two main ways to achieve a high concentration

Table I. Aqueous concentration of sisomicin as a function of time after subconjunctival injection

Sample no.	Time after injection (minutes)	Aqueous concentration (mg/l)
1	18	1.3
2	24	1.6
3	30	3.7
4	36	4.5
5	44	8.7
6	50	14.2
7	57	15.4
8	78	16.4
9	122	12.7
10	154	11.7
11	258	10.5
12	270	9.0
13	360	6.8
14	490	5.9
15	660	5.2
16	710	3.3
17	977	2.4
18	1203	0.9
19	1434	0.0
20	1464	0.0

Fig. 1. Aqueous penetration of sisomicin (20 mg/0.4 ml) after subconjunctival injection.

of a drug in the cornea and anterior chamber in the presence of an epithelial corneal defect: topical administration of a forte preparation and subconjunctival injection. Subconjunctival antibiotics circumvent the tissue barrier presented by the conjunctival and corneal epithelia and effect rapid entry of the drug into the aqueous humour and longer bioavailability.¹⁴⁻¹⁵ This is mainly because antibiotics delivered by other routes do not attain therapeutically effective concentrations in the aqueous humour, either due to inadequate penetration of the blood-aqueous barrier as when given systemically¹⁶⁻¹⁸ or, when the corneal epithelium is intact, as when they are given in the form of topical drops.¹⁹⁻²⁰ The aqueous penetration of several antibiotics has been documented,^{16-19, 21-23} but that of sisomicin remains unexplored. In the present study penetration of sisomicin into the anterior segment of the eye was estimated by measuring the aqueous humour concentration achieved by the drug as a function of time.

Our study showed that the subconjunctival administration of sisomicin delivered significant levels of the antibiotic into the aqueous humour. Within half an hour of injecting the drug, its level in the aqueous was sufficient to cover the minimum inhibitory concentration of *Staphylococcus aureus* (Table II).⁴ The reported minimum inhibitory concentration for *Pseudomonas aeruginosa* is 0.25 mg/l.⁴ At the peak drug titre attained at the 78 minute interval (approximately 1.15 hours), the aqueous concentration of 16.4 mg/l is 65 times the minimum inhibitory concentration of *P. aeruginosa*. Therapeutically effective levels

Table II. Minimum inhibitory concentrations (MIC) for sisomicin^{4,6}

Organism	MIC (mg/l)
<i>Staphylococcus aureus</i>	0.125
<i>Klebsiella pneumoniae</i>	0.125
<i>Escherichia coli</i>	0.5
<i>Pseudomonas aeruginosa</i>	0.25
<i>Proteus</i> (indole-negative)	0.15
<i>Proteus</i> (indole-positive)	0.15

Table III. Comparative data for aqueous penetration of various aminoglycosides

Reference	Year	Model	Antibiotic	Dose (mg)	Aqueous peak concentration (mg/l)	Bioavailability (hours)
Litwack et al. ¹⁶	1969	Rabbit	Gentamicin	20	16.3	2
Golden and Coppel ¹⁷	1970	Rabbit	Gentamicin	20	12.0	24
Kuming and Tonkin ³²	1974	Rabbit	Gentamicin	20	12.6	8
Uwaydah and Fairs ³³	1976	Rabbit	Tobramycin	10	6.70 (A) ^a	Not studied
Gorden and Cunningham ¹⁸	1982	Human	Tobramycin	10	16.5 (RIA)	8
Desai ¹⁹	1990	Human	Tobramycin	20	22.35 (RIA)	20
Orr et al. ²¹	1985	Human	Netilmicin	12.5	36.0	8
				25.0	38.0	9
Present study	1990	Human	Sisomicin	20	16.4	20

Key: RIA, radioimmunoassay; A, average.

^a Fixed interval.

of sisomicin against this organism are bioavailable in the aqueous up to 1203 minutes (20.05 hours). Choosing the minimum inhibitory concentration of *Pseudomonas* as a reference, it follows that in an anterior segment infection produced by these organisms, subconjunctival sisomicin injections must be spaced at approximately 24-hour intervals (or once a day) to maintain a therapeutically effective concentration of the drug in the aqueous humour.

There is good experimental evidence that the corneal level of antibiotics is considerably and consistently higher than the aqueous humour level at all times following a subconjunctival injection.²⁴⁻²⁶ Therefore, the high aqueous peak of sisomicin observed in the present study suggests that the drug must be available in much higher concentrations in the cornea, as it must negotiate this tissue barrier during the process of diffusion into the anterior chamber. For surface infections of the globe, sisomicin therefore has a favourable bioavailability. Significant changes in drug absorption can be brought about by the status of the cornea itself. Various corneal pathologies may either enhance or impede drug penetration into the anterior chamber. Corneal scarring will obstruct drug penetration into the cornea and then into the aqueous humour.²⁷ On the other hand, a disrupted epithelium and an inflamed or vascularised cornea would be expected to increase drug uptake. The present study documents the penetration of sisomicin in non-inflamed human eyes. In the event of keratitis or uveitis the drug levels found here are expected to be exceeded.

In recent years topical antibiotic delivery with fortified eyedrops and loading doses has gained acceptance in the treatment of corneal infections. Therefore, comparison of aqueous titres obtained with these modes of delivery and with subconjunctival injection should be interesting. Experimental studies with gentamicin have demonstrated that a fortified topical solution has very poor aqueous penetration in normal, uninfamed eyes.²⁸ But in inflamed eyes its concentration is considerably higher and reaches double the aqueous peak of subconjunctivally injected sisomicin obtained in the present study.²⁹ However, whatever convenience eyedrops offer in their administration is offset by the serious disadvantage of unreliable penetration, poor patient compliance and especially in the case of aminoglycosides, epithelial toxicity.^{30,31}

We compared the peak aqueous levels achieved with sisomicin with those of two other aminoglycosides – gentamicin and tobramycin—given subconjunctivally.^{16,19} A similarity in the peak aqueous concentration is evident between sisomicin and gentamicin (Table III).¹⁶ The sisomicin peak is also similar to that of tobramycin sulphate used in half standard dose (10 mg/0.5 ml)¹⁸ but considerably lower than levels obtained with a standard 20 mg/0.5 ml dose.¹⁹ A peak aqueous level of 22.34 mg/l was obtained at 2 hours with tobramycin in the latter study.¹⁹ A lower peak in the present study can be explained by the fact that the microbiological assay technique employed detects only the bioactive drug, whereas the radioimmunoassay utilised in the latter study¹⁹ measures both the active drug and its metabolites.

The comparative data (Table III) on the bioavailability of subconjunctivally injected antibiotics show that aminoglycosides, with the exception of netilmicin,²¹ have a relatively prolonged bioavailability (varying from 18 to 24 hours) compared with antibiotics such as cephaloridine.³⁴ The longer bioavailability is probably due to the fact that aminoglycosides bind avidly to melanin pigment of ocular tissues,³⁵ in contrast to cephaloridine which does not. This binding, which is freely reversible, allows a sustained release of the biologically active antibiotic into the anterior chamber.³⁶ The results of the present study conform to this pattern of bioavailability. Toxicity studies of sisomicin are not available in the literature. Netilmicin, a semi-synthetic aminoglycoside produced by the N-1 alkylation of its parent compound, sisomicin,³⁷ has been shown to have a retinal toxic threshold level of 100 µg after intravitreal injection.³⁸ If we assume a comparable threshold for sisomicin, the present study shows that such high levels of the antibiotic do not penetrate into the anterior chamber even at the peak titre. In view of sisomicin's broad range of antimicrobial activity, its efficacy against gentamicin-resistant strains of certain microbes and the excellent intraocular penetration achieved in the aqueous humour, we are of the opinion that sisomicin may be of considerable value in treating ocular infections and local sepsis following surgery, and for prophylaxis in cataract surgery.

Key words: Aqueous clearance, Bioavailability, Half-life, Pharmacokinetics, Sisomicin, Subconjunctival injection.

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