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Ocular distribution of aspirin and salicylate following systematic administration of aspirin to rabbits

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Abstract—The distribution of aspirin and salicylate 30 min after the intravenous administration of different doses of aspirin has been investigated in the rabbit eye. HPLC enabled a rapid and sensitive determination of both substances. A considerable dose-dependent penetration into all ocular tissues was observed with both aspirin and salicylate. Aspirin concentrations were higher than in plasma in all ocular tissues with the exception of the lens. These results show that an unhydrolysed drug may have a direct local effect by acetylating lens protein or other ocular constituents.

Aspirin has been reported to prevent cataract formation in man (Cotlier et al 1983). Various mechanisms have been proposed to explain this, some do not necessarily involve the presence of unhydrolysed aspirin in ocular tissues and could be attributed to salicylate, its hydrolysis product (Mizushima 1968; Sharma & Cotlier 1982; Van Heyningen & Harding 1986). A recently suggested mechanism involves acetylation of lens proteins; acetylation may prevent those non-enzymatic reactions such as glycosylation, carbamilation and steroid-binding which lead to the formation of high molecular weight protein aggregates that cause the development of opalescence (Bucala et al 1985; Crompton et al 1985; Rao et al 1985). This mechanism implies penetration of aspirin into the eye. It is therefore necessary to determine salicylate and aspirin levels separately. Although salicylate behaviour has been thoroughly studied, the presence of unhydrolysed aspirin in ocular tissues has not be investigated. Our aim has been to determine the extent of penetration of aspirin into the eye, in addition to that of salicylate, after intravenous administration, and its relation to the administered dose.

Materials and methods

Groups of five male New Zealand white rabbits, 2–3 kg, were injected via the marginal ear vein with aspirin as its tris-(hydroxymethyl) aminomethane salt. The animals were stunned and killed by bleeding and the blood samples collected into chilled heparinized test tubes containing 5 mg mL^{-1} potassium fluoride. The eyes were immediately enucleated and the aqueous humour of both chambers collected with a tuberculin syringe. Other eye fluids and tissues were collected or dissected, weighed and individually processed according to a modified method described for plasma (O'Kruk et al 1984). Plasma and ocular tissues were first added to 5 mg mL^{-1} potassium fluoride (25%) to prevent aspirin hydrolysis. Tissues were homogenized without, for vitreous humour, or after the addition of 500 μL distilled water for lens, cornea, iris and ciliary bodies, and retina with choroid. Plasma and tissues were added to 40 μL of a 30% w/v perchloric acid solution containing the internal standard 3,4,5-trimethoxybenzaldehyde (TMB), 800 μL methanol HPCL grade, vortexed for 2 min and centrifuged at 10 000 *g* for 10 min. The supernatant was then filtered through membrane filters of 0·45 μm nitrocellulose and aliquots of filtrate were injected into the chromatographic system consisting of a LKB 2150 HPLC pump, a Rheodyne 7125 injector and a Lichrosorb RP-18 (5 μm) 125 × 4 mm column (Merck). The effluent was monitored at 235 nm using a LKB 2151 variable wavelength monitor; quantification was accomplished with a LKB 220 integrator. The mobile phase consisted of a mixture of 0·1% potassium dihydrogen phosphate buffer and methanol (35%) and the final pH 3·3. The flow rate was 1 mL min^{-1}. Under these chromatographic conditions there was a good resolution of the three peaks in all the tissue samples without interfering peaks. The analysis was completed in 13 min. Fig. 1 shows a typical chromatogram of a corneal extract.

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The sensitivity of the method was such that 300 ng of aspirin and salicylate could be measured in 1 g of tissue.

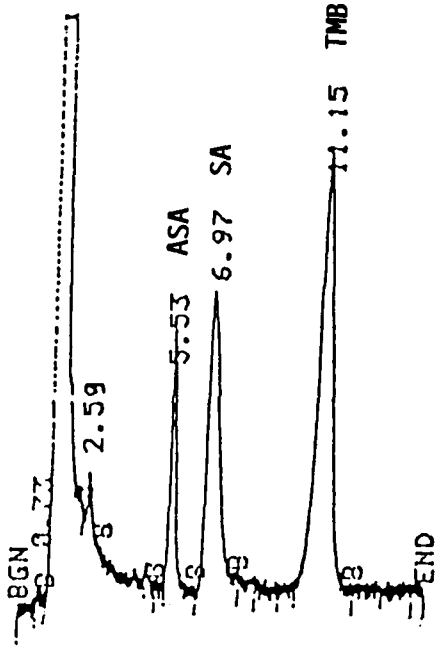

FIG. 1. Chromatogram of an extract of cornea, 30 min after the administration i.v. of 200 mg kg^{-1} of aspirin. ASA: aspirin. SA: salicylate. TMB: 3,4,5-trimethoxy-benzaldehyde (internal standard).

Results

As shown in Table 1, intravenous injections of increasing doses of aspirin resulted in a dose-related increase of aspirin and salicylate concentrations in plasma. The concentration of unhydrolysed drug was 2–3% that of salicylate. At that time a notable dose-dependent penetration of all ocular tissues by both drugs occurred. The highest concentrations of salicylate, about 20% of those in plasma, were found in vascularized tissues, iris, ciliary bodies and retina; lower levels, approximately 5–10% of those of plasma, were found in the aqueous humour, cornea and vitreous humour; the lowest level was in the lens. The concentrations of aspirin, although lower than those reached by salicylate were higher in all ocular tissues than in plasma with the exception of the lens. Moreover, the dose/concentration curve was steeper in all ocular tissues than in plasma; this behaviour was particularly marked in the aqueous humour, cornea and lens.

Table 1. Concentrations (g^{-1} or mL^{-1}) of acetylsalicylic acid (ASA) and salicylic acid (SA) in ocular tissues and in plasma of rabbits treated i.v. with different doses of aspirin and killed 30 minutes after the administration. Mean ± s.e. In parentheses is the number of animals.

	50 mg kg^{-1} (5)		100 mg kg^{-1} (5)		200 mg kg^{-1} (5)	
	ASA	SA	ASA	SA	ASA	SA
Plasma	3·9 ± 1·05	115·4 ± 18·57	6·0 ± 2·28	236·0 ± 12·40	10·6 ± 2·40	504·9 ± 58·52
Aqueous humour	2·7 ± 0·13	5·9 ± 0·40	8·0 ± 0·47	22·0 ± 4·59	32·6 ± 4·30	68·2 ± 2·61
Vitreous humour	4·3 ± 0·07	7·5 ± 0·25	9·1 ± 0·28	22·1 ± 1·27	19·6 ± 4·2	53·6 ± 10·4
Lens	—	0·5 ± 0·09	0·6 ± 0·09	4·0 ± 0·64	1·7 ± 0·3	10·1 ± 2·2
Retina	3·3 ± 0·34	21·3 ± 2·20	8·4 ± 0·45	55·1 ± 2·92	18·2 ± 6·30	119·6 ± 24·31
Cornea	4·4 ± 0·33	6·0 ± 0·45	10·4 ± 0·63	14·2 ± 0·86	37·7 ± 10·43	51·2 ± 7·41
Iris and ciliary bodies	3·1 ± 0·01	19·1 ± 0·03	9·3 ± 0·12	58·1 ± 0·78	21·3 ± 5·6	132·7 ± 18·6

Discussion

Our results show that aspirin, like salicylate, rapidly penetrates the eye suggesting a direct local effect. The plasma levels of aspirin obtained after 30 min were similar to those found in man 10–20 min after oral administration of 650 mg of aspirin (Rowland et al 1972).

The higher eye/plasma concentration ratio for aspirin than for salicylate may be due to protein binding, since salicylate has a much greater affinity for albumin (Davison & Smith 1961), and to the higher pK (Seed 1965). The fact that the dose-concentration curve is steeper in the eye than in plasma with both drugs, a feature shared by systemically administered drugs (Gardner 1987), strongly supports the theory of a penetration into the eye by diffusion.

Although aspirin concentrations in the lens are much lower than those capable of preventing lens opacification in-vitro (Bucala et al 1985), our results are in agreement with the suggested mechanism involving acetylation of lens proteins, if their slow turnover is considered along with long-term aspirin therapy in the subjects in epidemiological studies. However, many other possible mechanisms may play roles in the cataract-protective activity of the drug. The physical and chemical condition of the constituents of lens-surrounding areas, like aqueous humour, have a marked effect on the lens itself. Our results show that acetylation of these constituents may occur and may determine effects similar to those suggested in the lens. Acetylation may also cause an increase in the capacity of ocular proteins to bind substances involved in cataract development; in fact, this increase in binding proteins following acetylation, has been observed for plasma proteins (Pinckard et al 1973).

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