COMMUNICATIONS

ACTION OF ATROMID-S (CLOFIBRATE) ON INTRA-OCULAR PRESSURE

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CHANGES in the levels of the blood lipids during attacks of acute closed-angle glaucoma were studied by Hanisch, Blumenfeld, and Hegedüs (1966), who found that there was a rise in the concentration of the non-esterified fatty acids during the attack. Accordingly Orbán, Hanisch, and Vereb (1966) treated this condition with a single dose of 1.5 g. Atromid-S (clofibrate) and claimed that the attack was relieved in eight out of ten patients. Later, Cullen (1967) reported briefly that a single oral dose of 1.5 g. Atromid-S lowered the intra-ocular pressure in five of ten patients with chronic simple and secondary glaucoma; the normal intra-ocular pressure was said to be unaffected. A further short report from Orbán (1968) again claimed successful treatment of acute closed-angle glaucoma but gave evidence of a reduction in intra-ocular pressure in a proportion of cases of chronic closed-angle, open-angle, and secondary glaucoma.

Atromid-S (clofibrate; ethyl 2-(p-chlorophenoxy)-2-methyl propionate) is an antilipaemic drug which has been used in patients with coronary heart disease and raised serum lipids. It tends to have a corrective effect on a variety of disorders of coagulation and potentiates the action of anticoagulants. It has also been used in the treatment of various other conditions including hyperlipidaemia with xanthomatosis (Borrie, 1964) and exudative diabetic retinopathy (Cullen, Ireland, and Oliver, 1964).

The purpose of this paper is to report the effect of a single oral dose of 1.5 g. Atromid-S on sixteen patients with chronic glaucoma in whom the tensions were not fully controlled. One normal subject was also observed, but is not included in this report, his ocular tensions having been unaffected by clofibrate. In order to be certain that the patients were absorbing and excreting Atromid-S while they were being observed, the urines of four patients were examined for the glucuronide conjugate of chlorophenoxyisobutyric acid (CPIB), the form in which Atromid-S is excreted in the urine.

The effect of intramuscular injections of Atromid-S on the intra-ocular pressure in six rabbits was also observed.

Methods and Material

(1) Clinical Trial

Of the sixteen patients studied, nine had simple glaucoma, six chronic closed-angle glaucoma, which was bilateral in three, and one pigmentary glaucoma. The patients were seen on two

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consecutive days, and had their ocular tensions measured by Goldmann applanation tonometry five times during each day, as follows:

- 1st reading between 9.30 a.m. and 10 a.m.
- 2nd reading between 11 a.m. and 11.30 a.m.
- 3rd reading between 12 noon and 12.30 p.m.
- 4th reading between 2 p.m. and 2.30 p.m.
- 5th reading between 4.30 p.m. and 5 p.m.

Although the times of the readings varied slightly from patient to patient, care was taken that, for the individual patient, readings were taken at corresponding times on the two days of the test. If the patients were on any other treatment, this was continued throughout the trial, miotics being instilled or acetazolamide taken at the same time on both days. All applanation readings followed the standard procedure, the median of three readings being recorded; they were all taken on the same instrument and by the same observer.

The first day provided a series of control readings giving an indication of the diurnal variation in intra-ocular pressure. On the second day each patient was given 1.5 g. Atromid-S orally immediately after the first applanation reading. At the end of each day a sample of urine was taken from some patients for spectrophotometric evidence of excretion of Atromid-S, for measurement of pH, and for investigation of carbonic anhydrase inhibitory activity.

After the test, five patients were put on Atromid-S, one capsule three times a day, for periods varying between 2 and 4 weeks.

(2) Examination of the Urine

(a) *Spectrophotometric Analysis for Excretion Products of Atromid-S.*—The urines of four patients (Cases 13, 14, 15, 16) were examined for excretion products of Atromid-S. All four patients had samples of urine collected 6½ hours after taking Atromid-S, one (Case 15) had another sample taken 24 hours after, and another (Case 16) had a sample taken 2½ hours after Atromid-S.

Atromid-S (clofibrate) is the ethyl ester of CPIB; after oral administration it is readily absorbed and rapidly hydrolysed by tissue and serum esterases to free CPIB, which is excreted almost entirely as its water-soluble glucuronide conjugate. After hydrolysis with dilute alkali the free acid is formed and this can be extracted with an iso-octane-ethanol mixture. Spectrophotometric analysis of the extract shows absorption maxima in the ultra-violet at 280 and 226 mμ, their measurement allowing calculation of the concentration of the excretion product in the urine.

The urine samples were treated with 1 per cent. acetic acid by volume and stored overnight at 4°C. The CPIB content was estimated according to the method described in the "Atromid-S Review, 1966" issued by the Pharmaceuticals Division of I.C.I., Ltd. A urine "blank" was prepared by mixing 4 ml. urine with 1 ml. water. A hydrolysed urine sample was prepared by adding 1 ml. N sodium hydroxide to 4 ml. urine and heating the mixture at 70°C. for 15 minutes in a water bath. After allowing the hydrolysed urine to cool, 1-ml. samples of the "blank" and of the hydrolysed urine were placed in 10-ml. glass-stoppered tubes. To each was added 0.5 ml. 3 N hydrochloric acid and 5 ml. of an iso-octane-ethanol mixture (95 per cent. trimethyl pentane, 5 per cent. ethanol). The tubes were stoppered and well shaken and the layers then separated by centrifuging. The ultra-violet absorption spectra of the extracts were determined in 1-cm. silica cuvettes, using the Unicam SP 500 Spectrophotometer over a range of wavelengths from 300 to 210 mμ. The concentration of chlorophenoxyisobutyric acid in the hydrolysed urine samples was calculated by multiplying the optical density at 226 mμ by 187, giving the concentration in μg./ml.

(b) *Measurement of pH.*—Samples of urine were collected from six patients (Cases 11–16) and the pH of each sample was measured immediately after collection using an E.I.L. direct reading pH meter.

(c) *Estimation of Carbonic Anhydrase Inhibitory Activity.*—This estimation was based upon the indicator method for determining carbonic anhydrase activity by Roughton and Booth (1946) which depends on measuring the time taken for the pH of a barbitone buffer to change by a certain amount when a saturated solution of carbon dioxide in water is added at 0°C. 3 ml. of the barbitone buffer were diluted with 1.7 ml. distilled water and 6 drops bromothymol blue solution were added. The reaction was catalysed by the addition of 0.3 ml. of a 1/100 dilution of laked blood. In order to test for inhibition of this catalysis, the effect of a 0.3-ml. sample of urine collected on the second day of the test was compared with that of a similar sample taken on the first day. The end-point was determined by direct visual matching against a standard buffer (pH 6.3) containing bromothymol blue.

(3) Effect on Intra-ocular Pressure in Rabbits

In a series of six rabbits, three selected at random were given 625 mg. Atromid-S intramuscularly daily for 4 days. Ocular tensions were measured with a hand-held applanation tonometer and the observer did not know which animals had received the drug. Tensions were measured in the morning on the first day and the fourth day, and in the morning and afternoon on the second, third, and fifth days. The treated animals were injected after the morning tonometry on the second, third, fourth, and fifth days.

At the end of the experiment the mean for each set of applanation readings was calculated for all eyes of the treated animals and compared with the mean reading of all eyes in the untreated animals. These mean readings were converted into mm. Hg using the calibration for the rabbit eye (Hallman, 1967).

Results

(1) Intra-ocular Pressure in Glaucoma Patients

Since the hypotensive action of a drug is usually more obvious the higher the initial intra-ocular pressure, the eyes studied were divided into two groups in this respect. Group A consisted of seventeen eyes in which the tension exceeded 30 mm. Hg at some time during the two days of the trial; the remaining fifteen eyes in which the tensions did not reach 30 mm. Hg constituted Group B. The results are given in some detail together with the diagnoses and treatment in Tables I and II (pp. 798 and 799).

In a further analysis the intra-ocular pressures on the control day were compared with those at the corresponding times on the second day of the trial; these results are shown graphically for all the patients (Group A + Group B) in Fig. 1 (overleaf) and for the eyes with higher tensions (Group A) in Fig. 2 (overleaf). The value printed on the ordinate is the intra-ocular pressure on the second day minus the intra-ocular pressure at the corresponding time on the first day; any hypotensive effect of Atromid-S should appear on this graph, therefore, as a progressive tendency for the points to fall below the zero line with the passage of time. It is quite clear that there is no such tendency.

When individual patients were considered, it seemed possible that there was some evidence of a hypotensive effect in both eyes of Patient 1 (though this was not maintained after long-term administration of Atromid-S), while less certain reductions of pressure were recorded in Patients 2, 8, and 15.

In the five patients on treatment with Atromid-S for 2 to 4 weeks, there was no evidence of a fall in intra-ocular pressure; two of these patients had chronic closed-angle glaucoma and the remaining three had simple glaucoma.

FIG. 1.—Intra-ocular pressure in all glaucoma patients (see text).

FIG. 2.—Intra-ocular pressure in Group A patients only.

(2) Urine

(a) *Excretion Products of Atromid-S.*—Of the four patients from whom the urine was tested for excretion products of Atromid-S, two had tensions above 50 mm. Hg, and one between 40 and 50 mm. Hg, while the remaining one had tensions of up to 30 mm. Hg. The results for these four patients are given in Table III (opposite), and the absorption spectra of the urinary specimens taken $2\frac{3}{4}$ hours and $6\frac{1}{2}$ hours after administration of Atromid-S in Case 16 in Fig. 3 (opposite). These results illustrate that Atromid-S was being absorbed and excreted by these patients during the time that their intra-ocular pressures were being recorded.

TABLE III
ESTIMATION OF URINARY EXCRETION PRODUCTS
AFTER AN ORAL DOSE OF ATROMID-S

Case No.	Total CPIB in Urine (μg./ml.) at:		
	2½ hrs	6½ hrs	24 hrs
13	—	209.4	—
14	—	142.1	—
15	—	209.4	198.2
16	56.7	155.1	—

FIG. 3.—Absorption spectra of urinary specimens in Case 16, A 2½ hours, and B 6½ hours, after administration of Atromid-S.

(b) *pH*.—The pH of the urine was measured in six patients (Cases 11 to 16). There was no consistent change, the pH being lower after Atromid-S in three patients, higher in two, and unchanged in the remaining one.

(c) *Carbonic Anhydrase Inhibition*.—This was tested in fifteen patients (all except Case 1). In the urines of Patients 2 and 12, who were on Diamox, there was evidence of a substantial inhibition of carbonic anhydrase activity, as would be expected. In ten of the remaining thirteen patients, the reaction proceeded more slowly with the urine specimen obtained after Atromid-S as compared with that obtained on the first day; in the other three patients the reverse was found. It was noted that in all cases the urine obtained on the first day caused an appreciable reduction in the rate of reaction.

Although the solubility of Atromid-S in water is undoubtedly low, it was found that, on shaking up Atromid-S with distilled water and then separating by centrifuging, the aqueous phase gave spectrophotometric evidence of a very low content of Atromid-S. This solution was tested for inhibition of carbonic anhydrase activity and some suggestion of a very weak action was obtained. The latter, however, was considerably less than that obtained with a 0.1 per cent. solution of Diamox.

(3) Intra-ocular Pressure in Rabbits

The results are shown graphically in Fig. 4 (p. 799). The mean tensions for both groups were higher on the first day of the experiment and show some evidence of a diurnal variation, but there was no significant difference in the level of tension between the treated and untreated animals.

TABLE I
RESPONSES OF INTRA-OCULAR PRESSURE TO ORAL ATROMID-S
Group A—17 Eyes—Tensions 30 mm. Hg or over at some time during 2-day Atromid Trial

Case No.	Diagnosis	Treatment	Day	Ocular Tension			
				9.30–10.00 a.m.		4.30–5.00 p.m.	
				Right	Left	Right	Left
1	CCAG	Miotics and Diamox	1	18	18	30	31
			2	23	20	21	21
2	CSG	Miotics, neutral adrenaline, and Diamox	1	20		22	
			2	20		18	
4	CSG	Miotics	1		24		24
			2		28		23
6	Pigmentary glaucoma	Miotics	1	35		32	
			2	30		35	
9	CSG	None	1	38	34	29	28
			2	34	32	30	29
10	CCAG	Miotics	1		22		23
			2		25		25
11	CSG	None	1	30	30	19	19
			2	26	26	19	19
13	CCAG	None	1	47	44	34	36
			2	54	54	45	42
14	CSG	Miotics and neutral adrenaline	1	42	34	42	28
			2	38	32	48	32
15	CSG	None	1	28		26	
			2	28		23	
			3	30		—	
16	CSG	None	1	40	32	38	34
			2	38	33	44	34

CCAG = Chronic closed-angle glaucoma.
CSG = Simple glaucoma.

Discussion

When this group of patients is considered as a whole, it is quite clear that there was no evidence of an action of Atromid-S upon their intra-ocular pressures. It is also highly doubtful whether it had any such action in individual cases of this series. Our results have failed, therefore, to confirm those of previous observers.

This failure to find an effect cannot be attributed to deficient absorption of Atromid-S under the conditions of our experiment because spectrophotometric analysis of the urine demonstrated the presence of excretion products, an observation all the more significant since three of the four patients tested had considerably raised tensions. A second possibility is that the duration of observation in the main part of the clinical trial may not have been long enough to produce evidence of a hypotensive effect, but against this explanation one has to set the known facts of the rate of elevation of the serum concentration of

TABLE II
RESPONSES OF INTRA-OCULAR PRESSURE TO ORAL ATROMID-S
Group B—15 Eyes—Tensions below 30 mm. Hg during 2-day Atromid Trial

Case No.	Diagnosis	Treatment	Day	Ocular Tension			
				at 9.30–10.00		at 4.30–5.00	
				Right	Left	Right	Left
2	CSG	Miotics, neutral adrenaline, and Diamox	1 2		20 20		18 14
3	CCAG(R) AAC (L) with PI	Miotics R and L	1 2	18 16	15 14	18 20	15 14
4	CSG	Miotics	1 2	18 19		18 18	
5	CCAR (R) AAC (L) with PI	Miotics (R) PI (L)	1 2	16 22	14 17	22 23	16 18
6	Pigmentary glaucoma	Miotics	1 2		27 22		22 26
7	CSG	None	1 2	22 20	19 20	25 23	24 23
8	CCAG	Miotics	1 2	24 28	22 25	25 22	22 24
10	Suspected CCAG		1 2	18 15		15 15	
12	CSG	Miotics, neutral adrenaline, and Diamox	1 2	22 16	19 15	20 24	17 24
15	CSG	None	1 2 3		24 23 25		22 19 —

CCAG = Chronic closed-angle glaucoma.

AAC with PI = Acute angle-closure treated by peripheral iridectomy.

CSG = Simple glaucoma.

Atromid-S (Thorpe, 1962, 1964) and also our own observations on five patients who showed no response after 2 to 4 weeks of continuous therapy. The third factor to be taken into consideration is that the composition of our group of cases differed substantially from that reported by Orbán and others (1966), most of whose patients had acute closed-

FIG. 4.—Intra-ocular pressure in rabbits (see text).

angle glaucoma; also two out of five of the cases reported by Cullen (1967) as responding satisfactorily to Atromid-S were unilateral and therefore presumably cases of secondary glaucoma, a condition not represented in our series.

From the strictly therapeutic point of view our results indicate further that Atromid-S cannot be expected to be of any great benefit in controlling the ocular tensions in difficult cases of chronic primary glaucoma.

Finally, it has been suggested (Orbán, 1968) that any action that Atromid-S may have on the intra-ocular pressure could be explained in terms of the inhibition of carbonic anhydrase, as is the case with acetazolamide. In this connexion it is interesting to note that specimens of urine from the majority of our patients showed evidence of a very slight inhibitory activity. The significance of this observation is not clear, however, in view of the fact that the reaction used for revealing this inhibition was also slowed by specimens of urine obtained before Atromid-S had been administered. This would seem, therefore, to be a non-specific action, and the significance of the findings after Atromid-S remains in doubt.

Summary

The action of Atromid-S on the ocular tensions of sixteen patients with various types of chronic glaucoma was investigated. No significant effects were observed although analysis of the urine in some patients proved that the substance had been absorbed.

Intramuscular injections of Atromid-S did not influence the ocular tension of normal rabbits over a period of 4 days.

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