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Experimental pharmacological study of moroxybrate, a hypolipidaemic, antithrombotic, antiatheromatous agent

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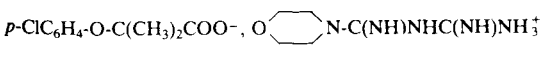
Moroxybrate, a drug obtained by the reaction of clofibric acid with moroxydine, a biguanide with practically no effect on carbohydrate metabolism, has been examined for its effects on blood lipids, fibrinogen, in-vitro platelet aggregation, and also on experimental atherosclerotic lesions obtained by feeding the rabbits a diet containing 1% cholesterol. At the same dose, moroxybrate has an effect at least equal to those of clofibrate and moroxydine alone, on the lipidic parameters and haemostasis in rat and rabbit. The combination of these actions together with an effect on the prevention of experimental atherosclerosis lesions in the rabbit suggests moroxybrate should be examined for its effects in ischaemic cardiovascular disease.

Moroxybrate (I) is a salt resulting from the reaction of chlorophenoxyisobutyric acid (clofibric acid), a cholesterol-lowering agent, which is also known to decrease platelet adhesiveness and to increase plasma fibrinolytic activity (Cotton & Wade 1969), with moroxydine (anhydrobis(betahydroxyethyl)-biguanide), which is claimed to have an antiviral activity, a slight effect on carbohydrate metabolism and to be a plasminogen activator (Robertson et al 1972; Hedner 1979; Tengborn et al 1982).



I

The product, obtained in stoichiometric proportions, is a crystalline white powder, soluble in water, with a well-defined melting point.

We have investigated the effect of moroxybrate on lipidaemia, clotting factors, platelet aggregation in rats and rabbits, and atheromatous lesions in rabbits. Its effects were compared with those of clofibrate and moroxydine in rats receiving a normal lipid or high lipid diet, and in rabbits receiving a sequential treatment allowing the development of an experimental aortic atherosclerosis.

MATERIALS AND METHODS

Experimental protocols

Rats fed a normal diet. 60 male Sprague-Dawley rats, 250 g, in 4 groups (n =15) had: group 1 normal diet (controls), group 2, normal diet + moroxybrate (35 mg day^{-1}/rat) given in the food, group 3, normal diet + clofibrate (35 mg day^{-1}/rat) given in the food and group 4, normal diet + moroxydine (35 mg day^{-1}/rat) given in the food, for one month.

Rats fed a high lipid diet. 60 male Sprague-Dawley rats, 250 g, in 4 groups (n = 15) were given a diet of composition (weight %): casein 23, glucose 22, starch 6, minerals 7, vitamins 1, butter 35.

These animals received the following treatment : group 1 controls, group 2 diet + moroxybrate (35 mg day^{-1}/rat), group 3 diet + clofibrate (35 mg day^{-1}/rat), group 4, diet + moroxydine (35 mg day^{-1}/rat). This experiment lasted one month.

Study on hyperlipidaemic and atherosclerotic rabbits. 20 male, crossed wild rabbits, 2·5 kg, received a normal diet over one month, and a daily subcutaneous injection of 1 mg adrenaline. The following month, rabbits were fed a diet containing 1% cholesterol. The animals were divided into 4 groups (n = 5): group 1 controls, group 2 moroxybrate (35 mg day^{-1}/rabbit), group 3 clofibrate (35 mg day^{-1}/rabbit), group 4 moroxydine (35 mg day^{-1}/rabbit). In the next three months, the rabbits were fed a normal diet, but still received the drug as indicated. This study lasted five months.

Methods

The following serum parameters were measured: (i) serum free cholesterol and cholesteryl esters (Enzymatic kit Boehringer), (ii) serum HDL-cholesterol (Enzymatic kit Boehringer modified), (iii) triglycerides according to Kessler & Lederer (1966), (iv) glycaemia by the potassium ferricyanure technique

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adapted to Technicon according to Hoffman (1937), (v) blood urea by the diacetyl-monoxyurea technique adapted to Technicon according to Marsh et al (1965), (vi) protidaemia by Folin reagent according to Lowry et al (1951) adapted to Technicon, (vii) transaminases SGPT (Mérieux reagent), (viii) fibrinogen according to Clauss (1957), (ix) clotting time according to the Howell technique (1912), (x) in-vitro measurement of platelet aggregation with ADP according to the Born Technique (1962) (the concentrations of ADP used were 15 μmol and 7·5 μmol; the measured parameter was the surface under the curve); (xi) cephalin kaolin by the technique of Langdell et al (1953).

The data controlled were: liver weight, the degree of hepatic steatosis (quoted from 0 to 3 according to the intensity and always evaluated by the same person), and a histological study of the aorta at three levels: origin—thoracic—and abdominal aorta. The elastic layers were coloured by means of an ethanolic solution of orcein, and the basic coloration was obtained by using the haematoxylic eosin method, and a modified Masson's trichrom method.

Statistical evaluations

A comparison of the means of the four groups of animals was performed by variance analysis (F test).

RESULTS

Haemostasis

The effect of moroxybrate was to diminish platelet aggregation, as measured by the area under the curve. The effect was stronger than with its two components and was present in rats on normal diet and those on the high diet (Table 1).

In rabbits, the platelet aggregation after 5 months of treatment was decreased in animals treated with moroxybrate and clofibrate compared with controls and animals treated with moroxydine (Table 1).

A decrease in blood fibrinogen was observed after treatment with moroxybrate in the three experimental models used. This decrease was higher with moroxybrate than with clofibrate (Table 2). The 'Howell time' and the 'cephalin kaolin test' were not modified by the treatment in any experiment.

Lipidic parameters

Rats on normal lipid diet. A decrease of cholesteryl ester levels was observed after treatment with moroxybrate and clofibrate, the free cholesterol being lower than after the other treatments. The HDL-cholesterol was also reduced after moroxybrate and clofibrate (Table 3).

Rats on high lipid diet. A large reduction in free cholesterol and cholesteryl esters was observed for the moroxybrate and clofibrate groups. The HDL-cholesterol remained identical with moroxybrate and increased after clofibrate (Table 3).

Effects on rabbits. No significant modification in total cholesterol and HDL cholesterol was observed, but a decrease in triglycerides occurred after moroxybrate (Table 4).

Table 1. Platelet aggregation (6 animals in each group of rats and 5 animals in each group of rabbits) results of surface under the curve (mean ± s.d.). Comparison by variance analysis (F test).

	Moroxybrate	Control	Clofibrate	Moroxydine	Variance analysis
Rats on normal diet					
ADP 15·0 μM	22·4 ± 4·8	30·5 ± 6·2	25·5 ± 1·8	26·0 ± 4·2	$P < 0·05$
ADP 7·5 μM	11·8 ± 3·1	19·3 ± 5·1	14·5 ± 5·7	14·5 ± 2·5	$P < 0·05$
Rats on high lipid diet					
ADP 15·0 μM	24·4 ± 6·1	38·3 ± 7·2	29·3 ± 4·8	24·9 ± 2·0	$P < 0·02$
ADP 7·5 μM	14·4 ± 1·6	16·0 ± 3·0	18·1 ± 1·1	12·7 ± 0·8	$P < 0·05$
Atherosclerotic rabbits					
ADP 15·0 μM	19·1 ± 3·0	24·5 ± 4·1	18·8 ± 2·8	24·6 ± 3·1	$P < 0·05$
ADP 7·5 μM	11·8 ± 1·2	14·7 ± 1·8	12·1 ± 0·9	14·6 ± 2·0	$P < 0·05$

Table 2. Blood fibrinogen in the 3 experiments. Results in g L^{-1} of plasma (mean of 9 animals for the rat experiments and on 5 animals for the rabbit experiment). Comparison by variance analysis (F test).

	Moroxybrate	Control	Clofibrate	Moroxydine	Variance analysis
Rats on normal diet	1·47 ± 0·04	1·91 ± 0·07	1·59 ± 0·02	1·98 ± 0·04	$P < 0·05$
Rats on high lipid diet	1·96 ± 0·04	2·16 ± 0·05	2·12 ± 0·09	1·90 ± 0·09	$P < 0·05$
Atherosclerotic rabbits	2·06 ± 0·18	3·83 ± 0·31	2·78 ± 0·13	2·82 ± 0·39	$P < 0·01$

Table 3. Blood lipid levels in rats on normal (N) and a high lipid (H) diet, (9 animals in each group). Comparison by variance analysis (F test).

		Moroxybrate	Controls	Clofibrate	Moroxydine	Variance analysis
Free cholesterol g L^{-1}	(N)	0·08 ± 0·02	0·10 ± 0·01	0·06 ± 0·01	0·08 ± 0·01	N.S.
	(H)	0·11 ± 0·01	0·20 ± 0·01	0·11 ± 0·01	0·18 ± 0·01	$P < 0·01$
Cholesteryl esters g L^{-1}	(N)	0·26 ± 0·04	0·37 ± 0·01	0·22 ± 0·03	0·32 ± 0·04	$P < 0·05$
	(H)	0·22 ± 0·02	0·28 ± 0·01	0·22 ± 0·01	0·29 ± 0·01	$P < 0·05$
HDL cholesterol g L^{-1}	(N)	0·30 ± 0·01	0·36 ± 0·01	0·29 ± 0·01	0·38 ± 0·01	$P < 0·01$
	(H)	0·25 ± 0·04	0·23 ± 0·01	0·38 ± 0·05	0·25 ± 0·04	$P < 0·02$

Table 4. Serum lipids in atherosclerotic rabbits (5th month), (5 animals in each group). Comparison by variance analysis (F test).

	Moroxybrate	Controls	Clofibrate	Moroxydine	Variance analysis
Total cholesterol g L^{-1}	0·66 ± 0·15	0·74 ± 0·15	0·69 ± 0·04	0·54 ± 0·10	N.S.
HDL cholesterol g L^{-1}	0·17 ± 0·04	0·22 ± 0·04	0·17 ± 0·01	0·15 ± 0·03	N.S.
Triglycerides g L^{-1}	0·86 ± 0·18	1·36 ± 0·10	1·22 ± 0·15	1·36 ± 0·14	$P < 0·05$

Table 5. Liver weight and steatosis level in rats on a high lipid diet, (9 animals in each group). Comparison by variance analysis (F test).

	Moroxybrate	Controls	Clofibrate	Moroxydine	Variance analysis
Liver weight (g)	16·75 ± 1·21	13·09 ± 0·56	18·49 ± 0·61	16·01 ± 0·86	$P < 0·05$
Degree of steatosis (%)	2·22 ± 0·22	1·0 ± 0·22	2·66 ± 0·16	2·88 ± 0·11	$P < 0·02$

The other biological parameters (glycaemia, urea, protides, transaminases) were not modified.

Morphological study

The liver weight and the steatosis of rats receiving the high lipid diet were higher with the three drugs than in the control group (Table 5).

Aortic atheromatous lesions were macroscopically smaller in the group treated with moroxybrate compared with the three other groups (Table 6). Moreover, the histological examination of aortae showed that the animals treated with moroxybrate had fewer atheromatous lesions (degree of lipidic infiltration, number of foam cells and degree of sclerosis) than animals from the two other treated groups.

Table 6. Aortic atheromatous lesions in rabbits (from 0 to 3), (5 animals in each group). Comparison by variance analysis (F test) $P < 0·05$.

Moroxybrate	0·70 ± 0·122
Controls	1·40 ± 0·254
Clofibrate	2·00 ± 0·316
Moroxydine	1·80 ± 0·374

DISCUSSION

Moroxybrate was synthesized with a view to combining the effects of clofibrate and moroxydine on lipid metabolism and haemostasis. It was necessary to verify if the anticipated effects had been achieved. With platelet aggregation, treatment with moroxybrate induced a response superior to that of clofibrate or moroxydine alone; this can be seen in both the rat and the rabbit. Similarly, the effect on blood fibrinogen was greater with moroxybrate. The effects of moroxybrate on blood lipids resembled those obtained with clofibrate.

From the results, a synergistic effect is apparent, since the product is a 1:1 (w/w) combination of clofibrate with moroxydine, and was given at the same dose as its constituents. Moroxybrate was active at several levels implicated in the atherosclerotic process. The work of Pickart (1981) emphasized the importance of blood fibrinogen as a major factor in blood and plasma viscosity in the microcirculation and in platelet and red cell aggregation in the appearance of atheromatous lesions. Thus, the known effects of biguanides on haemostasis (Isacsson & Nilsson 1972) and serum lipids (Marquié

1978; Tengborg et al 1982), and the effects of clofibrate on blood lipid parameters and fibrinolysis are potentiated by moroxybrate. The observed effect of moroxybrate on several risk factors of atherosclerosis could explain the reduction in atheromatous lesions in cholesterol-fed rabbit.

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