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Hypocholesterolemic agents—VI. Serum cholesterol lowering activity of β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors in rats

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Correlation between atherosclerosis and serum cholesterol levels has been discussed for many years. In order to lower serum cholesterol levels (and presumably the risk of atherosclerotic complications), drugs and modification of diet have been investigated. Since the enzyme β-hydroxy-β-methylglutaryl CoA reductase (EC 1.1.1.34) (HMG-CoA reductase) has been identified as an early physiological rate-controlling step in cholesterogenesis [1, 2] inhibition of this step would possibly be effective in decreasing cholesterol synthesis. Earlier reports [3, 4] from these laboratories have dealt with inhibition *in vitro* of this enzyme in rat liver microsomes. The present paper is concerned with the effect *in vivo* of inhibitors of HMG-CoA reductase on serum cholesterol in rats. Since a significant effect on the normocholesterolemic animal may not be reflected rapidly in the serum level, the Triton hypercholesterolemic animal was chosen. It has been reported [5] that Triton administration increases both HMG-CoA reductase activity and incorporation of acetate into cholesterol *in vivo*. A screening test for hypolipidemic drugs based on the administration of Triton has been developed [6].

The chemicals used in this study were: Triton WR-1339, Ruger Chemical Co., Irvington, NJ; sodium methohexital, Eli Lilly & Co., Indianapolis, IN; Stable Cholesterol Reagent, Hycel, Inc., Houston, TX; and propylene glycol, Fisher Scientific Co., Fair Lawn, NJ.

1-(4-Biphenyl)pentyl hydrogen succinate (I) was synthesized as described elsewhere [7]. 1-(4-Biphenylyl)pentyl hydrogen 3-hydroxy-3-methylglutarate (II), 1-(4-biphenylyl)pentyl hydrogen fumarate (III), and 1-(4-biphenylyl)pentyl hydrogen maleate (V) were synthesized as previously described [3]. The preparation of 4-[1-(4-biphenylyl)pentyloxy]butyric acid (IV) has also been described elsewhere [8]. 2-(4-Chlorophenoxy)isobutyric acid (CPIB) was obtained in a 89 per cent yield by saponification of clofibrate.*

The effectiveness of hypocholesterolemic drugs was determined according to Paoletti [9] with minor modifications.

Male Sprague–Dawley rats of 250–260 g were obtained from Holtzmann Co., Madison, WI, and given free access to Purina laboratory chow and water for approximately 1 week prior to beginning the experiment. The rats were then fasted for 18 hr and the fasted weights were 220–280 g. A solution of Triton WR-1339 (69 mg/ml in 0.9% aqueous NaCl) was injected into the tail vein of each rat at a dosage of 225 mg Triton/kg body weight. Hypo-cholesterolemic agents (as sodium salts) were dissolved in 25% propylene glycol–water (v/v) and administered by i.p. injection. Triton control rats received an equal volume of the vehicle by i.p. administration. Eight hr after injection, the rats were anesthetised by i.p. injection of sodium methohexital (45 mg/kg weight). The chest cavity was opened, and blood was obtained directly from the heart. The blood was allowed to clot in the refrigerator, and the serum was analyzed for cholesterol by use of Stable Cholesterol Reagent [10] (a direct Libermann–Burchard reagent) without extraction. This method was quite satisfactory over the cholesterol range involved.

It may be seen from Table 1 that the administraton of Triton WR-1339 elevated serum cholesterol levels (from the normal value of about 70 mg/100 ml) at 8 hr. The simultaneous administration of any of our four half acid-esters of dicarboxylic acids (compounds I, II, III and V) and the ether analog (compound IV) decreased this elevation at a dose level of 40 mg/kg. The compounds were devoid of activity at 20 mg/kg. The solubility of the compounds in the vehicle precluded doses above the reported levels without introducing other factors, i.e. dissolution rates of the compounds. For purposes of comparison, the known hypolipidemic agent, CPIB [11], was included.

Comparisons of the results *in vivo* of this paper and the previously described results *in vitro* are shown in Table 2. Compounds I and IV exhibit equivalent activity *in vitro* and *in vivo*. The results *in vitro* suggest that inhibition of HMG-CoA reductase does not require the ester group. The results *in vivo* indicate that there is no significant ester hydrolysis during the 8-hr time period. Bizzi *et al.* [12] have previously reported a 39 per cent decrease in serum cholesterol with 100 mg/kg of compound I in a slightly different 18-hr test.

Compound II was previously reported to be seven times more active than compound I in the system *in vitro* [3]. Incorporation of the β-hydroxyl and β-methyl functions into the inhibitor affords compound II which more closely resembles the natural substrate, HMG-CoA. Thus, it was expected that compound II would be more active *in vivo* than compound I. This is, however, not the case. This finding presently cannot be explained; however, the possibility exists that the tertiary β-hydroxyl group of compound II undergoes elimination to form a variety of unsaturated products.

Compound III (*trans*) is approximately 70 per cent more active than compound V (*cis*) in the system *in vitro*. Paradoxically compound V is about 60 per cent more active *in vivo* than compound III. This reversal of comparative activity may be related to differences in metabolism, distribution and protein binding of the two isomers.

In this study, compounds I, III, IV and V were found to be about three times more active than CPIB on a weight basis *in vivo* (or about five times more active on a molar basis). [Although 40 mg/kg of compound III lowered serum cholesterol a mean of 12.1 per cent compared to a lowering of 17.3 per cent by 120 mg/kg of CPIB, the difference in lowering exhibited by these two treatments was not statistically significant ($P < 0.1$)]. In the system *in vitro*, the least active of our inhibitors reported, compounds I and IV, were nine times more active than CPIB.

In summary, we have demonstrated the hypocholesterolemic activity *in vivo* of a series of inhibitors of HMG-CoA reductase. All of these inhibitors are more active *in vivo* as hypocholesterolemic agents and more active as inhibitors of HMG-CoA reductase *in vitro* than CPIB.

* Ethyl 2-(4-chlorophenoxy)isobutyrate (clofibrate), Ayerst Laboratories, Inc., New York, NY.

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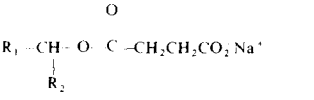
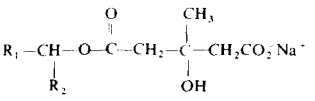
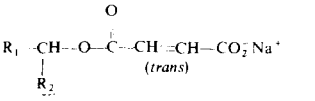
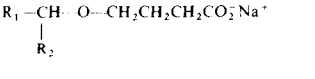
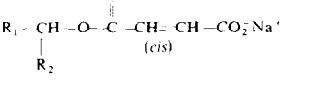
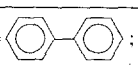
Table 1. Cholesterol lowering activity of HMG-CoA reductase inhibitors in Triton-induced hypercholesterolemic rats

Treatment	Structure*	No. of animals	Inhibitor dose (mg/kg)	Serum cholesterol† (mg/100 ml)	% Decrease†	P‡
Triton control		10		197 ± 3		
Compound I		10	20	193 ± 5	2.0 ± 1.3	NS
Compound II		10	20	194 ± 5	1.5 ± 1.1	NS
Compound III		10	20	184 ± 6	6.6 ± 2.1	NS
Compound IV		10	20	195 ± 4	1.0 ± 1.6	NS
Compound V		10	20	186 ± 7	5.6 ± 2.5	NS
Triton control		10		203 ± 4		
Compound I		10	40	172 ± 6	15.4 ± 2.8	<0.001
Compound II		11	40	184 ± 4	9.2 ± 1.7	<0.005
Compound III		11	40	179 ± 5	12.1 ± 2.4	<0.001
Triton control		20		213 ± 3		
Compound IV		10	40	180 ± 4	15.4 ± 2.1	<0.001
Compound V		20	40	177 ± 3	16.5 ± 1.4	<0.001
CPIB		11	120	174 ± 5	17.3 ± 2.3	<0.001
CPIB		11	240	152 ± 5	28.7 ± 2.5	<0.001

* $R_1 =$  ; $R_2 = CH_3(CH_2)_3^-$.

† Values are mean ± S. E. M.

‡ Student's *t*-test was applied to compare the test group to corresponding Triton control group. Non-significant differences are indicated by NS.

Table 2. Comparison of activity of inhibitors *in vitro* and *in vivo*

Compound No.	I/S_{50}^*	% Decrease (dose mg/kg)†
I	11‡	15.4 (40)
II	1.5‡	9.2 (40)
III	3‡	12.1 (40)
IV	11§	15.4 (40)
V	5‡	16.5 (40)
CPIB	100¶	17.3 (120)

* Molar ratio of inhibitor to substrate to produce a 50 per cent inhibition of rat liver microsomal HMG-CoA reductase, *in vitro*, as described [3].

† Per cent decrease in serum cholesterol in the Triton hypercholesterolemic rat.

‡ Data taken from Ref. 3.

§ Data taken from Ref. 8.

¶ Data obtained as described in Ref. 3.

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REFERENCES

1. V. W. Rodwell, J. L. Nordstrom and J. J. Mitschelen, *Adv. Lipid Res.* **14**, 1 (1976).
2. M. D. Siperstein and V. M. Fagan, *J. biol. Chem.* **241**, 602 (1966).
3. K. E. Guyer, S. G. Boots, P. E. Marecki and M. R. Boots, *J. pharm. Sci.* **65**, 548 (1976).
4. M. R. Boots, P. E. Marecki, S. G. Boots and K. E. Guyer, *J. pharm. Sci.* **65**, 724 (1976).
5. A. A. Kandutsch and S. E. Saucier, *J. biol. Chem.* **244**, 2299 (1969).
6. P. E. Schurr, J. R. Schultz and T. M. Parkinson, *Lipids* **7**, 68 (1972).
7. M. R. Boots, S. G. Boots, C. M. Noble and K. E. Guyer, *J. pharm. Sci.* **62**, 952 (1973).
8. S. G. Boots, M. R. Boots, K. E. Guyer and P. E. Marecki, *J. pharm. Sci.* **65**, 1374 (1976).
9. R. Paoletti, *Am. J. clin. Nutr.* **10**, 277 (1962).
10. *Hycel Cholesterol Determination Bulletin No. 5728*, p. 3. Hycel Inc., Houston, Texas (1974).
11. J. M. Thorpe, *Lancet* **I**, 1323 (1962).
12. L. Bizzi, C. Pozzatti, E. Ballardin and B. Silvestrini, *Boll. chim.-farm.* **100**, 855 (1961).