IN VITRO INHIBITION OF CHOLESTEROL UPTAKE IN HUMAN AND ANIMAL ARTERIES BY 7-KETOCHOLESTEROL

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Supported by grants from the Margaret W. and Herbert Hoover, Jr. Foundation, the Kenneth and Eileen L. Norris Foundation and The Council for Tobacco Research--U.S.A., Inc.

Received December 12,1974

Human and pig coronary arteries and rabbit aortas were perfused with pulsatile pressure in a modified Lindbergh apparatus with blood plasma obtained from the same species. Uptake of cholesterol by the arterial wall was measured using [^{3}H]-cholesterol as tracer. Percent distribution of synthesized lipid fractions was determined by thin-layer chromatography and liquid scintillation counting. Inhibition of cholesterol uptake by the arterial wall was studied by the addition of 7-ketocholesterol (concentrations of from 0.05 to 1 μmoles/ml in the perfusate). The addition of 7-ketocholesterol to the perfusate reduced cholesterol uptake by the vessel by an average of 90%. At concentrations of from 0.1 to 1 μmoles/ml of perfusate, 7-ketocholesterol inhibition remained unchanged. Inhibition was reduced at concentrations of ketocholesterol of 0.05 μmoles/ml. Inhibition was present in all species, and was not due to oxidation of cholesterol to 7-ketocholesterol in the perfusate. The results suggest inhibition of cholesterol uptake in the arterial wall by a competitive process.

In recent reports from this laboratory, it was found, using [^{14}C]-acetate, that human coronary arteries perfused *in vitro* do not form cholesterol but are able to synthesize other lipids (1, 2). However, using [^{3}H]-cholesterol as tracer, it was found that cholesterol was taken up by the arterial wall from the perfusion fluid. The view has been expressed by, among others, Walton *et al* (3) that cholesterol bound to lipoproteins is taken up by the arterial wall by a process of passive transfer. Others, however, are of the opinion that transfer into the arterial wall involves an active process with pinocytosis and related active transfer phenomena (4). The present report demonstrates that it is possible to inhibit the uptake of cholesterol by the arterial wall of several species by

an analog of cholesterol, 7-ketocholesterol (cholest-5-en-3β-ol-7-one). The results suggest competition between cholesterol and 7-ketocholesterol for binding sites within the vascular wall. The data also indicate a possible new and different approach to the prevention of cholesterol buildup in the arterial wall.

Methods

Pig coronary arteries and rabbit aortas used in the present investigation were removed within a few minutes after the death of the animals; they were dissected free of fat and connective tissue, and the side branches were ligated. The human coronary arteries obtained within 6 hours after death from 3 elderly individuals were similarly prepared. These vessels showed atherosclerotic involvement. Two separate pieces of cleaned arteries of similar diameter and of about 2 inches in length were used as experimental and control specimens. Two modified Carrel-Lindbergh pumps were employed to carry out the perfusion simultaneously for a period of 4 hours at 37°C, with a pulsatile fluid pressure of 120/80 mmHg at 80 cycles per minute (1). The basic perfusion fluid consisted of 250 ml of plasma pooled from the same species. Where pigs' arteries were used, the plasma was obtained from the same animal.

Preparation of Perfusate. A 100 mg sample of 7-ketocholesterol (Steraloids, Inc., Pawling, N.Y.) was dissolved in 5 ml of chloroform-methanol (2:1) mixture; the solution was equally divided into ten different test tubes, each of which was then placed on a rotovac to quickly evaporate the solvent in order to obtain a fine coating of 7-ketocholesterol on the inner wall. About 5 ml of plasma was then placed in each of the test tubes and sonicated in an ice bath two times for 30 seconds each at 20 kHz using a Biosonic III (Bronwill Scientific, Rochester, N.Y.) ultrasonic

system equipped with a microtip. After the sonication the contents of all the test tubes were pooled and brought to a volume of 125 ml with fresh plasma and incubated in a shaker bath for two hours at 37°C. In a separate procedure the free cholesterol in the lipoprotein fraction of 250 ml plasma was labeled with 250 μc of [^{3}H]-1,2-cholesterol as described earlier (1) and, in the experiments where lipid synthesis was also studied, 500 μC of [^{14}C]-2-sodium acetate were also dissolved in the plasma. One half (125 ml) of this plasma was mixed with plasma containing 7-ketocholesterol which was then used to perfuse one arterial specimen. The other half of the radioactive plasma was mixed with 125 ml of fresh plasma and this mixture was used to perfuse the control specimen. After the perfusion the perfusates and the arterial tissue were analyzed using a slightly modified procedure of Hashimoto, et al. (2)

Results

Results of the present investigation are presented in Table 1. A 7-ketocholesterol concentration of 1 μmoles/ml was generally used except in four experiments (Exps. 9, 10, 11 and 12), in which 0.1 or 0.05 μmoles/ml were used (Table 1). The arteries perfused in the presence of 7-ketocholesterol took up significantly less cholesterol compared to their corresponding controls ($p < .001$). Apparently there is no significant difference in the extent of inhibition of cholesterol uptake between 7-ketocholesterol concentrations of 1.0 and 0.1 μmoles/ml in the perfusate. However, the inhibition was reduced at 7-ketocholesterol concentrations of 0.05 μmoles/ml. No significant influence of 7-ketocholesterol on the synthesis of different lipid fractions by arterial tissue from [^{14}C]-acetate could be demonstrated ($p > 0.05$ in each case).

Table 1

Inhibition of cholesterol transport into arteries by 7-ketocholesterol

Expt. No.	Animal Species	PERFUSATE 7-keto-chol. conc. µmoles/ml	PERFUSATE Free chol. content µmoles/ml Control	PERFUSATE Free chol. content µmoles/ml Expt.*	TISSUE Free chol. content, µmoles/gm tissue Control	TISSUE Free chol. content, µmoles/gm tissue Expt.*	TISSUE Cholesterol uptake, nmoles/gm tissue Control	TISSUE Cholesterol uptake, nmoles/gm tissue Expt.*
1	Pig	1.00	0.63	0.73	3.1	3.0	221	49
2	"	1.00	0.43	0.43	3.8	3.8	257	34
3	"	1.00	0.43	0.38	3.8	3.8	127	9
4	"	1.00	0.22	0.22	4.6	4.5	139	76
5	"	1.00	0.41	0.38	5.4	4.0	588	36
6	"	1.00	0.29	0.28	3.6	5.0	666	12
7	"	1.00	0.39	0.41	8.5	6.1	297	18
8	"	1.00	0.34	0.30	4.9	4.7	362	49
9	"	0.10	1.06	0.99	5.6	4.5	2790	383
10	"	0.10	0.40	0.40	4.5	3.9	382	45
11	"	0.05	0.69	0.68	10.3	6.4	67	22
12	"	0.05	0.45	0.45	4.7	2.7	55	32
13	Rabbit	1.00	0.19	0.19	2.7	3.4	16	9
14	"	1.00	0.16	0.19	3.3	3.4	106	43
15	Human	1.00	0.68	0.75	100.8	52.5	189	16
16	"	1.00	1.03	0.98	55.4	11.0	63	24
17	"	1.00	0.83	0.83	7.5	35.7	289	12

* 7-ketocholesterol present in the perfusate

Analysis of arterial tissue perfused in the presence of 7-ketocholesterol revealed a distinct spot on the thin-layer plate corresponding to 7-ketocholesterol. This spot was absent in the control specimen. The color reaction described by Zak, et al

(5) for spectrophotometric determination of cholesterol did not produce any color with 7-ketocholesterol. This enabled the analysis of cholesterol in the presence of 7-ketocholesterol. The free and total cholesterol contents of the perfusates before and after the perfusion were found to be unchanged.

Discussion

In all the three species studied there was a significant decrease in cholesterol uptake by arteries in the presence of 7-ketocholesterol. This, together with the fact that 7-ketocholesterol could be detected in the perfused arteries suggests that this may be due to a competitive inhibition. The trichloroacetic acid precipitate of the perfusion fluid contained all the 7-ketocholesterol, which shows that it was bound to plasma proteins. The formation of 7-ketocholesterol-protein complexes has also been reported by Vendt (6).

Under certain conditions cholesterol is known to undergo auto-oxidation yielding 7-ketocholesterol as the main reaction product (7). It was therefore first suspected that the observed diminution in cholesterol uptake may be due to conversion of considerable quantities of cholesterol in the perfusate to 7-ketocholesterol catalyzed by the already-present 7-ketocholesterol. However, this possibility was excluded by the finding that free and total cholesterol contents of the perfusates before and after the perfusion remained the same within experimental errors ($\sim$5%).

It is difficult to propose a mechanism for the observed inhibition of cholesterol uptake. Such inhibition must depend on the nature of the transport of cholesterol into the arterial wall, which is not clearly understood (8, 9). If one assumes a physico-chemical transport process (9), cholesterol and 7-ketocholesterol may compete for identical binding sites. The fact that 7-ketocholesterol significantly inhibits cholesterol transport even at low concentrations

suggests that 7-ketocholesterol has comparatively high affinity for a large number of binding sites. The possibility must also be considered that 7-ketocholesterol competes for active transport mechanisms in the arterial wall, such as that furnished through pinocytosis. This is in line with the finding of Cantfort (10) that 7-ketocholesterol strongly inhibits the activity of the enzyme cholesterol-7α-hydroxylase *in vitro*. As the concentration of 7-ketocholesterol increases from 0.1 to 1.0 μmoles/ml there is no corresponding increase in inhibition to cholesterol uptake indicating a saturation effect (Table 1).

References

1. Morita, T., and Bing, R. J. (1972) Proc. Soc. Exptl. Biol. & Med. 140, 617-622.
2. Hashimoto, H., Tillmanns, H., Sarma, J. S. M., Mao, J., Holden, E., and Bing, R. J. (1974) Atherosclerosis 19, 35-45.
3. Walton, K. W., and Williamson, N. (1968) J. Atheroscler. Res. 8, 599-624.
4. Schwartz, S. M., and Benditt, E. P. (1972) Am. J. Path. 66, 241-264.
5. Zak, B., Dickenmann, R. C., White, E. G., Burnett, H., and Cherney, P. J. (1954) Am. J. Clin. Path. 24, 1307-1315.
6. Vendt, V. P. (1956) Vitaminy, Akad-Nauk Ukr S.S.R. 2, 30-39 (CA 52-7617d, 1958).
7. Bergstrom, S., and Wintersteiner, O. (1941) J. Biol. Chem. 141, 597-610.
8. Björkerud, S., and Bondjers, G. (1973) Nutr. Metabol. 15, 27-36.
9. Hashimoto, S., and Dayton, S. (1966) J. Atheroscler. Res. 6, 580-590.
10. Cantfort, J. V. (1972) Life Sci. 11 (Part II), 773-780.