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Inhibition of cholesterol synthesis reduces low-density-lipoprotein apoprotein B production without decreasing very-low-density-lipoprotein apoprotein B synthesis in rabbits

Agnes LA VILLE, Ray MOSHY, Peter R. TURNER, Norman E. MILLER and Barry LEWIS
Department of Chemical Pathology and Metabolic Disorders, St. Thomas' Hospital Medical School, London SE1 7EH, U.K.

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The kinetics of the apoprotein B (apo B) of very-low-density (VLDL; $d < 1.006$) and low-density (LDL; d 1.019–1.063) lipoproteins were studied in six rabbits by using radioiodinated homologous lipoproteins, before and during oral administration of mevinolin (5mg/kg per day), a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34), to explore the mechanism by which the drug reduces LDL synthesis. Before treatment LDL-apo B production greatly exceeded VLDL-apo B production in all animals, indicating that a large proportion of plasma LDL was derived from a VLDL-independent pathway. Five animals responded to mevinolin with a fall in plasma cholesterol (mean change -53%; $P < 0.01$). This was associated with a 66% decrease in LDL-apo B synthesis ($P < 0.05$). In contrast, VLDL-apo B synthesis was unaffected by mevinolin. Furthermore, in all but one animal the decrement in LDL-apo B synthesis was greater than the rate of VLDL-apo B synthesis before treatment, demonstrating that mevinolin had reduced the VLDL-independent production of LDL.

Hepatic triglyceride is secreted as a component of VLDL ($d < 1.006$). Subsequent hydrolysis of the triglyceride by endothelial-bound lipases converts VLDL to IDL (d 1.006–1.019), which is partly cleared from the circulation and partly converted to LDL (d 1.019–1.063) in the splanchnic bed (Turner *et al.*, 1981). In some circumstances a proportion of plasma LDL is produced by a process not involving VLDL (Janus *et al.*, 1980; Kesaniemi *et al.*, 1981; Goldberg *et al.*, 1983). Catabolism of LDL occurs partly by a receptor-mediated process in hepatic and peripheral cells (Goldstein & Brown, 1977).

The production of plasma lipoproteins requires the synthesis, intracellular transport and assembly of diverse lipid, carbohydrate and protein components. The major protein of VLDL, IDL and LDL is apo B. This is a structural component, which does not exchange between particles and is quantitatively conserved during the conversion of VLDL to LDL (Hammond & Fisher, 1971); it is also the recognition protein for the receptor-mediated catabolism of LDL (Goldstein & Brown, 1977).

There is little information on the degree to which apo B metabolism is influenced by the metabolism of cholesterol. Kovanen *et al.* (1981) reported that suppression of cholesterol synthesis in dogs by administration by mevinolin, a competitive inhibitor of HMG-CoA reductase (EC 1.1.1.34) (Alberts *et al.*, 1980), decreased the plasma concentration of LDL both by increasing the FCR of the lipoprotein and by reducing its rate of synthesis. The present investigation was undertaken to determine whether mevinolin diminishes LDL-apo B production by reducing VLDL-apo B synthesis, by reducing the percentage conversion of VLDL to LDL, or by decreasing the VLDL-independent production of LDL.

Abbreviations used: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; apo B, apoprotein B; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; FCR, fractional catabolic rate.

Materials and methods

Materials

Mevinolin was a gift from Merck, Sharp and Dohme, Rahway, NJ, U.S.A. $Na^{125}I$ and $Na^{131}I$ were obtained from Amersham International,

Amersham, Bucks., U.K. Sagatal (phenobarbitone sodium B.P., 60mg/ml) was purchased from May and Baker, Dagenham, Essex, U.K., and 1,1,3,3-tetramethylurea was purchased from Sigma Chemical Co.

Animals and experimental protocol

Male New Zealand White rabbits (*Oryctolagus cuniculus*) were maintained on Sg1 rabbit diet with vitamin C (Grain Harvested, Canterbury, Kent, U.K.) from weaning, and were studied between the ages of 6 and 12 months. Body weights were initially 2.88–4.58kg (mean 3.48kg). In all but one rabbit (no. 6), in which there was an increase of 0.57kg (19.8%), weights remained unchanged throughout (mean change +0.06kg, 2.9%).

Mevinolin was administered orally in bread. To train the animals to accept this preparation, the daily food allowance was reduced from *ad libitum* to 75g plus two plain commercial white bread cubes (measuring 2cm^3) 2 weeks before introducing the drug. Mevinolin (5mg/kg per day) was subsequently added to the bread cubes in ethanol (150μl per cube), the cubes being dried in air for 1h. For 25–32 days two such cubes were fed to each rabbit each morning, followed by the daily ration of Sg1 diet (increased to 100g to maintain body weight). Drug-impregnated bread cubes were prepared every 4 days and stored at 4°C.

VLDL-apo B and LDL-apo B metabolism was studied in each animal before and during mevinolin administration. Homologous ^{131}I-VLDL and ^{125}I-LDL (2–4μCi of each) were injected simultaneously into an ear vein. Venous blood samples (4ml) were then collected from the other ear at the following post-injection times: 10min, 1h, 1h 45min, 2h 30min, 5h, 7h, 9h and 24h.

Laboratory procedures

To obtain sufficient VLDL and LDL for radioiodination, starved rabbits were anaesthetized with Sagatal and blood was collected from the inferior vena cava into sterile tubes containing disodium EDTA (1mg/ml of blood). Plasma was separated and the VLDL and LDL fractions were isolated by sequential preparative ultracentrifugation (105000*g* for 16–20h) at densities of 1.006, 1.019 and 1.063g/ml in a Kontron 45.6 rotor (Turner *et al.*, 1981). VLDL was labelled with ^{131}I and LDL with ^{125}I by the ICl method (Janus *et al.*, 1980). Precipitation with trichloroacetic acid demonstrated that less than 2% of the radioactivity in the final preparations was present as free iodide. Of the radioactivity, 41% in the ^{131}I-VLDL and 67% in the ^{125}I-LDL was associated with apo B.

Isolation of VLDL and LDL from post-injection plasma samples was by preparative ultracentrifugation (Turner *et al.*, 1981). The apo B was precipitated with tetramethylurea and then resolubilized in NaOH (1mmol/l) (Turner *et al.*, 1981). We have previously shown that the recoveries of VLDL-apo B and LDL-apo B by this procedure average 91 and 83% respectively (Turner *et al.*, 1981). Radioactivity was measured in an LKB Ultragamma twin-channel counter, using the Flexiprogram (R) board. Counting time was 20min and the minimum count was 10000 (ensuring a counting error of less than 1%).

Calculations

For radioactivity, time curves of VLDL-apo B were mono-exponential for 7–9h, by which time 95% (mean) of the radioactivity had been cleared. The FCR of VLDL-apo B was therefore derived as 0.693/half-life. LDL-apo B radioactivity decayed as a double exponential, and was accordingly analysed using a two-pool model (Matthews, 1957). For measurement of VLDL- and LDL-apo B pool sizes, aliquots of the lipoproteins were pooled and concentrated 4-fold by recentrifugation. The apo B was then precipitated with tetramethylurea, delipidated and solubilized (Turner *et al.*, 1981), and then assayed (Lowry *et al.*, 1951) using bovine serum albumin as standard. Pool sizes were calculated as apo B concentration × plasma volume, the latter being 3.5% of body weight (Nuwayahid, 1979). Synthetic rates were then calculated as pool size × FCR/body weight.

Results

Results obtained for plasma cholesterol concentration, VLDL-apo B metabolism and LDL-apo B metabolism before and during mevinolin are presented in Table 1. Mevinolin substantially reduced the plasma cholesterol concentration in all but rabbit no. 6. This animal also differed in having a very low LDL-apo B concentration before treatment. In view of these findings, separate statistical analyses were performed including and excluding this animal.

Reduction of plasma cholesterol was associated with a marked decrease in the synthetic rate of LDL-apo B [mean change ($n = 5$) −66%], and with a large increase in the FCR of LDL-apo B (mean change +188%). LDL-apo B pool size was reduced on average by 80%. In contrast, no consistent changes were observed in the synthetic rate, pool size or FCR of VLDL-apo B.

Discussion

During the control period the rate of production of LDL-apo B exceeded that of VLDL-apo B in all animals. Although Hornick *et al.* (1983) failed to observe any direct secretion of LDL by perfused

Table 1. *Effects of mevinolin on plasma total cholesterol concentration, VLDL-apo B metabolism and LDL-apo B metabolism in six rabbits*

C, control period; M, mevinolin period. *P* values were calculated by paired *t*-test analysis; N.S., $P>0.10$.

	Plasma cholesterol (mmol/l)		VLDL-apo B						LDL-apo B					
			Plasma pool (mg)		Synthetic rate (mg/kg per h)		FCR (h^{-1})		Plasma pool (mg)		Synthetic rate (mg/kg per h)		FCR (h^{-1})	
Animal	C	M	C	M	C	M	C	M	C	M	C	M	C	M
1	2.43	1.10	0.58	2.09	0.10	0.13	0.63	0.23	25.8	8.8	0.47	0.17	0.07	0.08
2	2.02	1.00	0.65	0.38	0.16	0.10	0.73	0.92	13.5	0.4	0.29	0.03	0.06	0.24
3	2.14	0.98	0.69	0.36	0.06	0.04	0.31	0.32	9.1	0.3	0.23	0.01	0.08	0.17
4	2.05	1.00	1.91	1.27	0.14	0.12	0.35	0.37	28.4	4.3	0.52	0.16	0.08	0.15
5	0.90	0.37	0.62	1.39	0.09	0.43	0.51	1.12	10.9	1.6	0.38	0.30	0.13	0.51
6	1.70	1.73	0.45	0.96	0.05	0.23	0.32	0.82	2.9	2.6	0.13	0.30	0.13	0.39
Rabbits 1–6														
Mean	1.87	1.03	0.82	1.08	0.10	0.18	0.48	0.63	15.1	3.0	0.34	0.16	0.09	0.26
P	<0.005		N.S.		N.S.		N.S.		<0.05		0.05		<0.05	
Rabbits 1–5														
Mean	1.91	0.89	0.89	1.10	0.11	0.16	0.51	0.59	17.5	3.1	0.38	0.13	0.08	0.23
P	<0.01		N.S.		N.S.		N.S.		<0.05		<0.05		0.05	

rabbit livers, previous evidence for VLDL-independent production of LDL by some rabbits *in vivo* has been provided by the studies of Kushwaha & Hazzard (1978). A similar phenomenon has been documented in *Cynomolgus* monkeys (Goldberg *et al.*, 1983) and in humans with familial (Janus *et al.*, 1980) or polygenic (Kesaniemi *et al.*, 1981) hypercholesterolaemia.

In all but one rabbit, mevinolin substantially reduced the plasma cholesterol concentration, providing evidence for effective suppression of cholesterol synthesis. Since mevinolin is known to inhibit HMG-CoA reductase in hepatocytes, the major source of plasma VLDL, as well as in several peripheral tissues (Alberts *et al.*, 1980; Endo, 1981), the absence of any associated change in VLDL-apo B production suggests that the secretion of newly synthesized apo B in VLDL is independent of the rate of hepatic cholesterogenesis in rabbits.

In contrast, and in agreement with the results of Kovanen *et al.* (1981), the synthetic rate of LDL-apo B was greatly reduced in all five animals who responded to mevinolin with a fall in plasma cholesterol. Furthermore, in four rabbits the magnitude of the decrease in LDL-apo B synthesis greatly exceeded the rate of VLDL-apo B synthesis before treatment. Thus, the principal mechanism underlying the reduction of LDL production by mevinolin must have been a decrease in the VLDL-independent synthesis of LDL (or IDL) by the liver, although an additional effect on the percentage conversion of VLDL to LDL cannot be excluded.

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