ANTIMICROTUBULAR AGENTS INHIBIT THE DEGRADATION OF CHYLE CHOLESTEROL ESTER IN VIVO[1]

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SUMMARY

The effects of colchicin and vinblastin on the metabolism of intravenously injected cholesterol ester labelled chyle and chylomicrons were examined. Both agents delayed the hydrolysis of the cholesterol esters after their uptake by the liver. The data suggest, that the degradation of chyle cholesterol esters in the liver occurs by a mechanism depending on intact microtubular functions. The process does not require de novo synthesis of protein, since it was not inhibited by cycloheximide.

INTRODUCTION

During the metabolism of chylomicron triglyceride (1,2) cholesterol ester rich particles are formed (3). These are degraded by the hepatocytes (4-7). How this occurs is poorly understood, but it has been suggested, that the hydrolysis of the residual triglycerides (8) and of the cholesterol ester (6) may occur in the plasma membrane. The present study was undertaken to examine, whether the metabolism of chylomicron remnant particles by the hepatocytes requires intact microtubular functions. This hypothesis was based on the observations that antimicrotubular agents inhibit a number of transport processes involving granular or vesicular transport and membrane fusion (9). Although there is no evidence that the uptake of chyle cholesterol ester occurs by vesicular transport and hydrolysis in secondary lysosomes (6), other microtubule dependent mechanisms may be involved. For instance components of the chylomicron remnant particles might be incorporated into the hepatocytes by a process, similar to membrane fusion. The chylomicron cholesterol ester does not participate in exchange reactions with

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other lipoproteins and tissues and is rapidly hydrolyzed in the liver. It was therefore a suitable marker in experiments designed to test whether antimicrotubular agents affect the degradation of the chylomicron remnants.

METHODS

Male Sprague-Dawley rats were used in the fed state. Radioactive chyle was obtained from rats with thoracic duct fistulas (10), which were fed 50-200 μC [1-2-$^{3}H_2$]cholesterol and in some experiments also 10 μCi [^{14}C]oleic acid in 0.5-0.75 ml triolein, as described earlier (11). The distribution of radioactivity among particles with S_f > 400 and S_f < 400 was determined according to Minari and Zilversmit (12). Chylomicrons (S_f > 400) for injection were prepared by the same procedure. Intestinal VLDL[2] were prepared by centrifugation at 100,000 g for 25 hrs (d=1.006) after chylomicrons had been removed at 60,000 g for 60 min. Serum treated at 56°C for 30 minutes to inactivate the lecithin cholesterol acyltransferase was labelled with nonesterified [^{14}C]cholesterol as described earlier (13). All labelled substances were obtained from Radiochemical Centre. The radiopurity was 96 % or better on silica gel G thin layer chromatography in petroleum ether:diethyl ether:acetic acid 80:20:1.

Colchicin (British Drug Houses Ltd), cycloheximide (Sigma) and vinblastin sulfate (Lilly) were injected intraperitoneally in 0.9 % saline. Controls were injected with saline. 90-270 minutes later the chyle or lipoproteins were injected into the jugular vein under ether anesthesia. After different time intervals, the animals were killed by bleeding through aortic puncture. The extraction of lipids and isolation and determination of radioactivity of the different lipid classes is described elsewhere (14). Triglycerides were determined by gas liquid chromatography of the fatty acid methyl esters (15). Cholesterol was determined according to Zak et al. (16) after saponification of the lipid extract (17).

RESULTS AND DISCUSSION

In an experiment where colchicin treated and control animals were injected with doubly labelled ([^{3}H]cholesterol, [^{14}C]oleic acid) chyle, there was no significant differences between the groups in the removal of radioactive triglyceride or cholesterol from blood within 15 min (Table I). 30 min after injection the serum cholesterol radioactivity was higher in colchicin treated than in control rats, but the difference was not significant and amounted to a minor portion of the injected dose (Fig. 1). The radioactivity in the liver was similar in the two groups. Delayed clearance from blood may therefore not be the reason, why the time course for the hydrolysis of chyle cholesterol ester in the liver was slower after treatment with

[2] VLDL = very low density lipoproteins.

TABLE I. Effect of colchicin on the elimination of doubly labelled chyle lipoprotein from blood.

Group of animals	Total cholesterol radioactivity in liver (cpm)	Triglyceride radioactivity in liver (cpm)	Total cholesterol radioactivity per ml serum	Triglyceride radioactivity per ml serum (cpm)
Controls	13708 ± 1294	4866 ± 246	1507 ± 194	194 ± 48
Colchicin	14664 ± 1279	4946 ± 695	1616 ± 138	128 ± 21

Rats weighing 185-220 g were injected with 0.25 ml (7.4 mg lipid, 45.2 µg cholesterol) chyle from an animal fed [^{3}H]cholesterol and [^{14}C]oleic acid in triolein. The animals were killed 15 minutes later. The injected radioactivity was 29040 cpm ^{3}H of which 61 % was in cholesterol ester, and 18100 cpm ^{14}C, of which 86 % was in triglyceride. 93.3 % of the triglyceride- and 82.5 % of the cholesterol ester radioactivity was in particles with S_f > 400. The colchicin (1 mg/100 g body weight) was injected 3 hrs before the injection of the chyle. The figures are means ± S.E.M. of six observations.

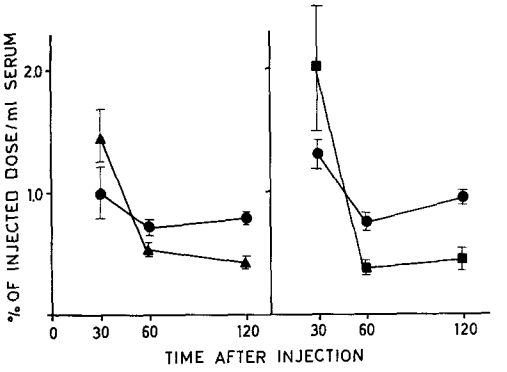

Fig. 1. Effects of colchicin and cycloheximide on the serum cholesterol radioactivity at different times after injection of cholesterol labelled chyle. Chyle from animals fed [^{3}H] cholesterol was injected intravenously into control animals and animals treated with 1 mg colchicin or 0.1 mg cycloheximide/100 g body weight. The animals weighed 185-285 g and controls and drug treated animals of each series were matched according to weight. The data are % of total injected dose [^{3}H]cholesterol found per ml serum as cholesterol plus cholesterol ester in the experiments described in Table I. ●—● control groups; ▲—▲ colchicin; ■—■ cycloheximide.

colchicin (Table II). As in earlier studies (4,5) the hydrolysis in the normal rats was almost completed within an hour after injection. In the colchicin treated animals about half of the radioactive cholesterol ester that had been taken up by

TABLE II. Effect of colchicin and cycloheximide on the time course of the chyle cholesterol ester hydrolysis in rat liver.

Time after injection (min)	% of liver lipid radioactivity in cholesterol ester		% of injected ^3H in liver lipids	
	0.9 % NaCl	Colchicin	0.9 % NaCl	Colchicin
30	25.0 ± 4.9	51.6 ± 6.3^{xx}	70.3 ± 6.0	62.8 ± 7.0 n.s.
60	10.7 ± 1.5	47.0 ± 4.2^{x}	66.0 ± 2.9	76.3 ± 3.1 n.s.
120	5.8 ± 1.3	24.2 ± 4.6^{xxx}	49.7 ± 2.8	70.5 ± 2.7^{xx}
	0.9 % NaCl	Cycloheximide	0.9 % NaCl	Cycloheximide
30	43.4 ± 5.4	39.0 ± 2.5 n.s.	64.7 ± 9.1	45.0 ± 5.7^{xxxx}
60	8.0 ± 1.4	11.9 ± 2.4 n.s.	60.8 ± 6.0	58.9 ± 5.9 n.s.
120	4.7 ± 0.5	8.1 ± 1.2^{xx}	44.4 ± 3.3	59.7 ± 3.2^{xxx}

Control animals, and animals that had been treated with colchicin (1mg/100 g body weight) 3 hrs earlier, or cycloheximide (0.1 mg/100 g body weight) 2 hrs earlier were injected with cholesterol labelled chyle. In the experiments with cholchicin the injected chyle contained 7.25 or 11.05 mg lipid, 33.3 or 75.2 µg cholesterol, 45100 or 111000 cpm [^3H]cholesterol of which 62 or 74 % was in cholesterol ester. In the experiments with cycloheximide the chyle contained 2.7-7.4 mg triglyceride, 33.5-60 µg cholesterol 51000-220000 cpm [^3H] cholesterol of which 44.6-74 % was in cholesterol ester. 73.7-82.5 % of the injected cholesterol ester radioactivity was in particles with $S_f > 400$. The significance of the differences was estimated by the t-test. $^{x}p < 0.001$, $^{xx}0.01 > p > 0.001$, $^{xxx}0.02 > p > 0.01$, $^{xxxx}0.1 > p > 0.05$, n.s. = significant. All values are means ± S.E.M. of six observations.

the liver remained unhydrolyzed even after 2 hrs (Table II). The delay of the hydrolysis was seen at a dose of 0.05 mg colchicin per 100 g body weight but was more marked if 0.5-1 mg/100 g was given (Fig. 2). The effect was thus seen in the dose range known to inhibit lipoprotein secretion by the liver in vivo (18). The increase in cholesterol ester radioactivity may not be due to increased cholesterol ester formation in the liver, since no increased esterification was noticed of [^{14}C]cholesterol that was injected as lipoprotein and entered the liver presumably by exchange processes (Fig. 2).

In the experiments with whole chyle (73.7-82.5 % of the radioactive cholesterol ester was present in chylomicrons ($S_f > 400$) and the smaller part in VLDL. When isolated chylomicrons and VLDL were injected, colchicin delayed the hydrolysis of the cholesterol ester of both lipoprotein classes. A larger part of the chylomicron cholesterol ester had, however, been hydrolyzed

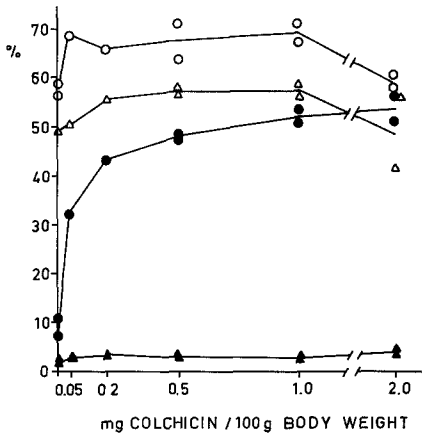

Fig. 2. Effect of increasing dose of colchicin on the hydrolysis of chyle cholesterol ester in rat liver. The colchicin was given intraperitoneally in 0.5 ml 0.9 % saline 2 hrs before the injection of [^{3}H]cholesterol labelled chyle (7.7 mg lipid, 94 µg cholesterol, 12500 cpm as cholesterol and 19100 cpm as cholesterol ester. 100 µl serum containing 30615 cpm non-esterified [^{14}C]cholesterol and negligible amounts of [^{14}C]cholesterol ester was injected simultaneously. The figure shows % of injected ^{3}H (O—O) and ^{14}C (Δ—Δ) in the liver, and the proportion of this radioactivity that was found as cholesterol ester. (●—● % of ^{3}H and ▲—▲ % ^{14}C in cholesterol ester.) One of two similar experiments is shown. Two duplicate samples (0.05 and 0.2 mg/100 g) were lost.

60 min after injection (Table III). This may be due to the quicker removal from blood of the chylomicron than of the VLDL cholesterol ester (19). The data thus indicate that the cholesterol ester of both lipoprotein classes is metabolized by colchicin sensitive mechanisms. The proportion of radioactivity that was present as cholesterol ester in the liver 60 minutes after injection of chylomicrons was increased also in rats that had been treated with vinblastin (Table III).

Cycloheximide had the same effects as colchicin on the time course of the plasma cholesterol radioactivity (Fig. 1). Somewhat more of the injected chyle cholesterol was still present in blood after 30 min. The serum radioactivity 60 and 120 min after injection was decreased by both drugs. This may be due to inhibition of the cholesterol secretion as lipoprotein from the liver (18,20). In the case of colchicin, the delayed hydrolysis would also decrease the transfer of radioactive cholesterol to plasma lipoproteins by exchange reactions.

TABLE III. Effects of colchicin and vinblastin on the metabolism of chylomicron and intestinal very low density lipoprotein cholesterol esters.

Lipoprotein class	% of injected dose in liver	% of lipid radioactivity in cholesterol esters in liver and in injected material	
Chylomicrons			
A. Injected material	-	55.1	
Control	69.8 ± 11.7	10.5 ± 1.8^{xx}	(n=3)
Colchicin	58.4 ± 3.0 n.s.	19.5 ± 1.4^{xx}	(n=3)
B. Injected material	-	63.2	
Control	52.4 ± 2.8	13.4 ± 3.1	(n=4)
Vinblastin	54.5 ± 4.1 n.s.	39.7 ± 3.2^{x}	(n=4)
VLDL			
Injected material	-	80.4	
Control	60.2 ± 3.0	15.5 ± 2.9	(n=3)
Colchicin	61.2 ± 0.9 n.s.	54.1 ± 1.5^{x}	(n=3)

In the experiments with colchicin 250 µl chylomicrons (1.39 mg triglyceride, 15.7 µg cholesterol) or very low density lipoproteins (0.290 mg triglyceride, 45.0 µg cholesterol) were injected intravenously in animals treated with 1 mg colchicin per 100 g body weight 3 hrs earlier and into controls injected with saline. The animals were killed 60 minutes later. The injected chylomicron radioactivity was 23150 cpm as cholesterol ester and 18760 cpm as cholesterol. The injected VLDL contained 251800 cpm as cholesterol ester and 60980 cpm as nonesterified cholesterol. In the experiments with vinblastin the injected chylomicrons contained 13.5 mg triglyceride 70 µg cholesterol, 28200 cpm as free cholesterol and 51800 cpm as cholesterol ester. The vinblastin sulfate (1 mg/100 g body weight) was given by intraperitoneal injection 90 min before the injection of the chylomicrons. $^{x}p < 0.001$, $^{xx}0.02 > p > 0.01$ according to t-test. Values are means ± S.E.M.
The effect of vinblastin on the metabolism of VLDL cholesterol ester was not examined.

Cycloheximide had no significant effect on the hydrolysis of chyle cholesterol esters (Table II). The proportion of the liver radioactivity in cholesterol ester, 120 min after the injection of the chyle, was slightly larger than in the control group, but this small difference was also seen in two experiments where nonesterified radioactive cholesterol was injected as lipoprotein. It may therefore be due to accumulation of newly synthesized cholesterol ester, rather than delayed hydrolysis of the chyle cholesterol esters. The data thus give no evidence, that an active protein biosynthesis is necessary for the degradation of chylomicron remnant particles. It is then also unlikely that the antimicrotubular agents act by inhibiting the intracellular migration of newly synthesized proteins which participate in the process.

In other experiments colchicin did not inhibit the hydrolysis in suspended hepatocytes of chyle cholesterol ester, previously taken up by the liver in vivo (Å.Nilsson, unpubl. obs.). This indicates that the drug does not directly inhibit the hydrolysis once the cholesterol ester is available to the hydrolytic enzymes. The present data then suggest that a colchicin sensitive transport function may be necessary for the interiorization of the cholesterol ester or its hydrolysis in the plasma membrane (6). The finding that vinblastin, which acts on microtubules by another mechanism than colchicin (9) also delays the hydrolysis of the chylomicron cholesterol ester (Table III) suggests that the action of colchicin is due to its antimicrotubular activity rather than to a direct effect on the plasma membrane (9,21).

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