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EFFECT OF CERIUM ON LIVER LIPIDS METABOLISM AND PLASMA LIPOPROTEINS SYNTHESIS IN THE RAT

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SUMMARY

After a single injection of Cerium chloride to female rats, liver triglycerides concentration increases sharply and plasma lipids decrease to about one half the initial level. In these conditions, the respiratory quotient of treated rats in vivo decreases by 20% indicating a lowered fatty acid oxidation. Incorporation of [3H]-oleate into liver lipids shows an important accumulation of newly synthetized triglycerides without any significant effect on phospholipids. Lipoproteins production estimated by [3H]-oleate and [14C]-leucine incorporation into plasma very low-, low- and high-density lipoproteins shows a 50% inhibition synthesis of lipoprotein.

INTRODUCTION

In spite of several studies (1-5), the mechanism through which Cerium chloride induces a triglyceride accumulation in the liver of female rats is not yet fully understood. Decreased oxidation of short chain fatty acids from adipose tissue (5) as well as decreased secretion of triglyceride-rich lipoproteins by the liver (8) could explain the development of the Cerium-induced steatosis. We have recently shown (9) that adrenalectomy which prevents catecholamine-mediated lipolysis in adipose tissue after Cerium injection to rats, prevents also hepatic triglyceride deposition, thus indicating that excessive FFA mobilization is essential to the development of fatty liver. Considering that 1) the FFA uptake by the liver is directly proportional to the plasma FFA concentration (10) and 2) FFA availability controls the

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ABBREVIATIONS: FFA: free fatty acids; VLDL, LDL and HDL: very low-, low- and high-density lipoproteins; b.w.: body weight; TCA: trichloro acetic acid.

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production of triglyceride-rich lipoproteins by the perfused rat liver (11), an increase of the VLDL secretion rate after Cerium injection to animals could be expected. Nevertheless, contradictory results have been obtained in previous studies where triglyceride secretion was measured with Triton WR 1339 injection : either decreased (4,8) or increased (12) triglyceride secretion has been described.

In order to clarify these conflicting results, lipoproteins secretion by the liver has been studied in control rats and in those receiving a Cerium chloride injection 12 and 24 hours before. In other animals, the respiratory quotient has been continuously recorded during 24 hours before and after Cerium administration.

MATERIALS AND METHODS

Animals : Female Wistar rats weighing 200-250 g receive under light ether anesthesia 0.5 ml of 155 mM sodium chloride (Control rats) or Cerium chloride solution (3 mg of Cerium, as element, per kg b.w.) into the femoral vein. As Cerium administration reduces food intake (5), Control and Cerium-treated rats were pair-fed. All animals were fasted overnight with free access to tap water before the sacrifice.
Metabolic studies : In order to determine the effect of Cerium on the synthesis of liver lipids as well as on the secretion of lipoproteins, we administered one hour before the sacrifice to each animal 10 µCi of [14C]-leucine (specific activity 50 µCi/mmol) and 50 µCi of [3H]-oleate (specific activity 153 mCi/mmol) into the femoral vein under light ether anesthesia. At the time indicated after saline or Cerium injection, animals were anesthetized with ether and blood was drawn in a heparinized syringe from the abdominal aorta.
Isolation of plasma lipoproteins : VLDL ($d<1.006$), LDL ($1.019<d<1.063$) and HDL ($d<1.21$) were isolated from plasma by preparative ultracentrifugation (13). Lipoproteins fractions were dialyzed during 20 hours against 155 mM sodium chloride and aliquots were removed for lipid extraction and quantitative determination of their protein moiety.
Analytical methods : Lipids were extracted from duplicate samples of plasma, lipoproteins and liver (14). Triglycerides (15), cholesterol (16) and phospholipids (17) determinations were carried out on aliquots of the lipid extracts. In order to study the incorporation of [3H]-oleate into plasma lipoproteins, aliquots of the lipid extract were spotted on thin layer silica gel plates. The plates were developed in petroleum ether, diethyl oxide, acetic acid, 90/30/1; v/v/v and the lipid identified after exposure to iodine vapor. The corresponding silica gel zones were scraped off into vials containing a liquid scintillation mixture and counted in a β-spectrometer.
The determination of [14C]-leucine incorporated into the protein moiety of plasma lipoproteins has been described (18). The liver and plasma lipoprotein protein content was determined (19). Respiratory quotient of rats was continuously recorded during 24 hours following Cerium injection (20).
Statistical methods : Unless specified, all the data are given as the mean ± SEM and evaluated statistically by the Mann-Whitney U-test.

TABLE 1. TIME COURSE STUDY OF RESPIRATORY QUOTIENT AFTER CERIUM.

Period (hrs)	2-8	8-14	14-20	20-24
Control rats	0.73 ± 0.02 (91.6 %)	0.73 ± 0.01 (91.6 %)	0.72 ± 0.01 (95.2 %)	0.71 ± 0.01 (98.9 %)
Cerium rats	0.78 ± 0.02* (73.7 %)	0.78 ± 0.03* (73.7 %)	0.77 ± 0.01* (77.2 %)	0.75 ± 0.01* (84.4 %)

All data are given as the mean ± SEM from 6 rats per group, determined and compared as described in MATERIALS and METHODS.
* : $p < 0.01$. Figures in parentheses represent the percentage of fatty acid oxidation.

RESULTS AND DISCUSSION

As indicated by the increase of respiratory quotient, the amount of lipid oxidized by the Cerium-treated rats decreases by 20% as early as 8 hours after the rare earth injection (Table 1). As the animals were kept fasted during all the study, we observe a constant decrease of the respiratory quotient in both groups of animals. Yet, the difference between Control and Cerium-treated animals remained constant. The decreased capacity of the rats to oxidize fatty acids *in vivo* constitutes the earliest Cerium-induced alteration of lipid metabolism. A decreased capacity of isolated liver mitochondria to oxidize octanoate *in vitro* has been described by others (6,7) one to six days after Cerium administration. Nevertheless, different rare earth added *in vitro* to normal rat liver mitochondria preparations failed to interfere with oxidative phosphorylation and ATP production (21).

A time course study of plasma following Cerium administration to female rats reveals 12 hours later an important decrease of triglycerides (39%), cholesterol (23%) and phospholipids (41%) concentrations (Fig.1). From 12 to 24 hours, triglycerides and cholesterol plasma concentrations decrease further, the phospholipids values remaining unchanged (Fig.1). Similar modifications of plasma lipids have also been described in animals treated with an equivalent dose of Praseodymium (8).

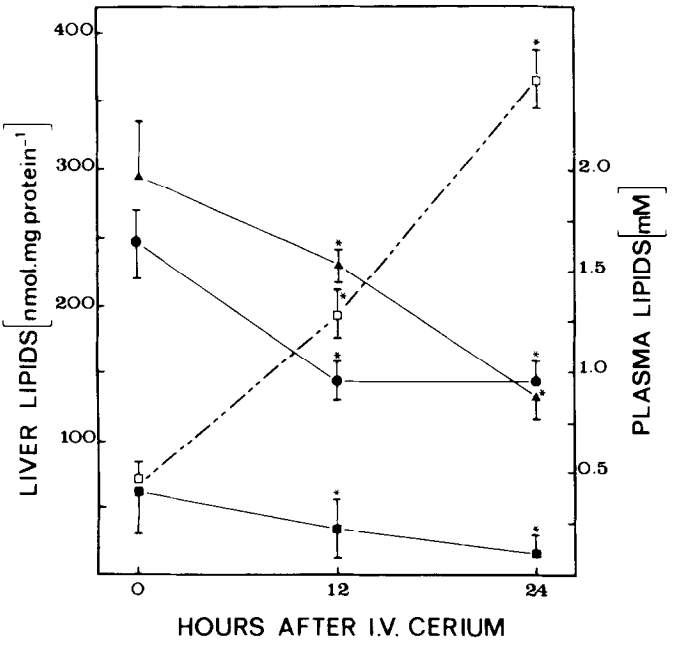

FIGURE 1. Time course study of liver triglycerides and plasma lipids after intravenous injection of Cerium. Liver: □, triglycerides (dashed line). Plasma: ■, triglycerides; ●, phospholipids; ▲, cholesterol (solid lines). Data represent the mean±SD from 6 experiments. Experimental conditions are described in MATERIALS AND METHODS. *: $p<0.01$

As shown in Table 2, the incorporation of [3H]-oleate into liver triglycerides increases 3 fold as early as 12 hours after Cerium administration. On the contrary, phospholipids synthesis seems unaffected. A similar situation is observed 24 hours after Cerium (Table 2).

In order to measure the contribution of a decreased lipid secretion to the accumultation of liver triglycerides after rare earth poisoning, we investigated the liver capacity to secrete lipoproteins. Although the use of the detergent Triton WR 1339 seems appropriate to measure the secretion rate of triglycerides (22), it induces important transfer of lipid and apoproteins between different classes of lipoproteins which makes inaccurate the determination of lipoproteins secretion rate by this method (23). Tn this respect, we have measured the secretion of VLDL, LDL and HDL in vivo after intravenous injection of radioactive precursors. The incorporation of [3H]-oleate into VLDL

TABLE 2. EFFECT OF CERIUM ON THE [3H]-OLEATE INCORPORATION INTO LIVER LIPIDS AND PLASMA LIPOPROTEINS.

	Triglycerides	Phospholipids
LIVER	(dpm/mg protein)	
Control rats	8023 ± 172	2219 ± 104
12 hrs after Ce	26858 ± 163*	2438 ± 122 (NS)
24 hrs after Ce	32048 ± 273*	2128 ± 97 (NS)
PLASMA LIPOPROTEINS	(dpm/ml plasma)	
VLDL		
Control rats	21936 ± 1116	2555 ± 250
12 hrs after Ce	7439 ± 1204*	2051 ± 591 (NS)
24 hrs after Ce	4636 ± 480*	1707 ± 680 (NS)
LDL		
Control rats	6912 ± 599	1658 ± 238
12 hrs after Ce	1534 ± 129*	1492 ± 91 (NS)
24 hrs after Ce	1340 ± 171*	2205 ± 60 (NS)
HDL		
Control rats	-	7404 ± 1089
12 hrs after Ce	-	4294 ± 381*
24 hrs after Ce	-	1364 ± 182*

All data are given as the mean ± SEM from 6 experiments per group, determined and compared as described in MATERIALS and METHODS.
* : $p < 0.01$; NS : not significant.

and LDL triglycerides is reduced respectively to 34 and 22% of the Control values 12 hours after Cerium injection (Table 2). Later on, a further decrease is observed and the secretion of these two lipoproteins does not represent more than 20% of the values observed in Control animals (Table 2). As was observed for liver phospholipids, the incorporation of [3H]-oleate into VLDL- and LDL-phospholipids is only slightly modified (Table 2). On the contrary, HDL phospholipids secretion represents 58 and 18% of the Control values respectively 12 and 24 hours after Cerium injection (Table2). A very low incorporation of [3H]-oleate could be detected in HDL triglycerides and thus is not reported.

As observed for VLDL and LDL lipids, their protein moiety is also affected by Cerium: Table 3 shows an important decrease of VLDL (50%) and LDL (42%) apoprotein concentration during the first 12 hours following the rare earth injection. During the rest of the study, VLDL and LDL apoprotein concentration remains steady (Table 3). Contrasting

TABLE 3. EFFECT OF CERIUM ON THE CONCENTRATION AND SYNTHESIS OF PLASMA APOLIPOPROTEINS.

		Control rats	12 hrs after Ce	24 hrs after Ce
VLDL	A (µg/ml)	30.2 ± 2.4	15.1 ± 0.6*	18.0 ± 2.5*
	B (dpm/ml)	85 ± 1	54 ± 3*	69 ± 1*
LDL	A (µg/ml)	116.5 ± 3.7	67.8 ± 5.3*	58.8 ± 2.4*
	B (dpm/ml)	188 ±10	112 ± 2	104 ± 5
HDL	A (mg/ml)	3.2 ± 0.2	2.9 ± 0.2 (NS)	1.8 ± 0.1*
	B (dpm/ml)	285 ±23	146 ±12*	72 ± 1*

All data are given as the mean ± SEM from 6 experiments, determined and compared as described in MATERIALS and METHODS. A : protein concentration and B : [14C]-leucine incorporation into lipoproteins.
* : $p < 0.01$; NS : not significant.

with VLDL and LDL, HDL apoprotein concentration does not decrease significantly during the first 12 hours after Cerium, but represent 57% only of the Control values after 24 hours (Table 3). The incorporation of [14C]-leucine into VLDL and LDL apoproteins parallels the decrease of their protein concentration (Table 3). On the contrary, the [14C]-leucine incorporation into HDL apoprotein decreases much more rapidly than its plasma concentration: 50 and 75% respectively 12 and 24 hours after Cerium (Table 3).

The different evolution of VLDL and LDL concentrations compared to that of HDL results more from the longer half-life of the HDL molecule than from the effect of Cerium on their respective synthesis rate. Cerium chloride administration results also in a decreased [14C] -leucine incorporation into liver and plasma TCA-precipitable proteins (results not shown). The mechanism of protein synthesis inhibition by Cerium remains to be elucidated: although this cation has a high affinity for DNA (24) which could result in an inhibitory effect on RNA synthesis, liver polyribosomes patterns are not modified by Cerium administration (12).

The inhibition of lipoproteins synthesis we have observed in this study can not explain totally the considerable accumulation of liver triglycerides since the lipoprotein secretion represents about 20% of the overall triglycerides synthesis by the liver. Then, it can be concluded that the major metabolic effect of Cerium is an excessive fatty acids availability to esterifying microsomal enzymes as a result of increased FFA influx and decreased mitochondrial oxidation. The 50% inhibition of lipoproteins secretion rate aggravates the liver triglycerides accumulation.

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