Triglyceride Alterations in Normal and Reticuloendothelial Stimulated Rats Following Carbon Tetrachloride[1,2]

N. R. Di LUZIO, Department of Physiology, University of Tennessee Medical Units, Memphis, Tennessee

Abstract

The administration of a single dose of carbon tetrachloride to normal rats was associated with a significant increase in liver triglyceride concentration and a pronounced reduction in plasma triglycerides. The evaluation of hepatic activity indicated that the removal of bromsulphalein from plasma was significantly impaired while phagocytic function remained unaltered. Animals with pronounced hyperplasia and hyperfunction of the reticulo-endothelial system induced by zymosan were less susceptible to carbon tetrachloride, manifesting a smaller degree of liver triglyceride accumulation and maintenance of a normal plasma triglyceride level. The possible significance of these findings are discussed.

PREVIOUS STUDIES have demonstrated that the intravenous administration to rats of the yeast cell wall fraction, zymosan, results in a selective proliferation and a hyperfunctional state of the reticuloendothelial system (RES) (1–4). The feeding of atherogenic diets to rats with an induced hyperfunctional state of the RES was also demonstrated to be associated with a decreased plasma and liver cholesterol ester accumulation, suggesting the participation of Kupffer cells, a major component of the reticuloendothelial system in the metabolism of cholesterol (3–5). The definition of the RES and the relationship of liver Kupffer cells to this system were previously discussed (6).

The cells comprising the RES have also been implicated in various areas of lipid metabolism (6,7) including the metabolism of triglycerides and fatty acids (8). Evidence has also been presented indicating a profound reaction and marked sensitivity of the RES of the rat to carbon tetrachloride administration (9). In an effort to define the involvment of the RES in other areas of lipid metabolism and to evaluate the sensitivity of newly formed reticuloendothelial (RE) cells to hepatotoxins, the present study was undertaken to determine the influence of a hyperfunctional state of the RES on triglyceride alterations occurring in experimental liver injury due to the administration of carbon tetrachloride.

Experimental Procedures

Reticulo-endothelial hyperplasia and hyperfunction was induced in female rats by the daily intravenous injection of 2 mg of zymosan per 100 g body weight for 6 days. Control rats received equivalent volumes of saline, the suspending medium for zymosan. The rats were fasted for 16 hr on the day following the last injection and carbon tetrachloride in a 1:1 mixture with mineral oil was administered by stomach tube, in the amount of 0.25 ml/100 g of body weight. Control rats received an equivalent volume of mineral oil. Six hours after intubation, blood samples were obtained from the aorta, and liver, spleen, and lung weights were determined.

Plasma free fatty acids (FFA) were determined by a modified Dole procedure (10). Liver and plasma triglycerides were determined by the Van Handel and Zilversmit method (11).

In an effort to correlate hepatic functional activity with hepatic lipid changes, phagocytic activity of Kupffer cells (3,4) was measured by the colloidal carbon technique 6 hr. after intubation. Sulfobromophthalein sodium (BSP) clearance (12) was used to evaluate the level of hepatic parenchymal cell activity. The removal of BSP from plasma was measured 5 min following the injection of 50 mg of BSP per kg of body weight 4 hr after the injection of colloidal carbon and 6 hr after carbon tetrachloride or mineral oil administration in 24 rats which were not previously injected with colloidal carbon.

Results

The oral administration of carbon tetrachloride significantly reduced the concentration of plasma triglycerides in the saline injected group (Table I). This reduction of approximately 60% was associated with a 4 fold increase in liver triglycerides. The plasma FFA, although decreased, did not differ significantly from control values.

The repeated intravenous injection of zymosan produced a 48% increase in liver weight and an increase in the weight of those organs, such as spleen and lung, which have relatively large RE cell populations.

The reduction in plasma triglyceride concentration following carbon tetrachloride was significantly inhibited in the RE hyperfunctional group. A reduced accumulation of liver triglyceride was also observed. The total liver triglyceride (liver weight × triglyceride concentration) ranged from 25.8 mg to 52.1 mg in the saline group which received mineral oil. The total liver triglyceride in the saline carbon tetrachloride-treated group ranged from 135 to 296 mg. In contrast, the total liver triglyceride in the zymosan group treated with carbon tetrachloride was significantly reduced, having a range of 73 to 158 mg. The experiment was conducted on three different occasions, and the decreases in total liver triglyceride in the zymosan carbon tetrachloride-treated rats when compared to the saline injected carbon tetrachloride-treated rats ranged from 37 to 52%. The administration of zymosan to normal rats has been previously demonstrated to produce no alterations in total liver triglycerides (5).

In an attempt to determine functional differences between the various groups, phagocytic activity, as determined by the intravascular removal of colloidal carbon was determined prior to an evaluation of BSP removal. The former is a test for RE function and particularly the hepatic Kupffer cells, while the latter is an index of hepatic parenchymal cell activity (13).

The removal rate of colloidal carbon, as denoted by its half time (t/2) in the saline mineral oil group was 8.6 min and was unaltered by carbon tetrachloride (Table II). The profound hyperplasia and hyperfunctional state of the RES induced by zymosan is readily noted by the pronounced increases in liver,

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TABLE I

Triglyceride Alterations Following Carbon Tetrachloride in Normal and Reticuloendothelial Stimulated Rats [a]

Group	No. rats	Body weight, g	Hematocrit, %	Organ weight, g			Plasma FFA mEq/L	Triglyceride		
				Liver	Lung	Spleen		Plasma, mg %	Liver, mg/g	Total liver, mg
Saline + mineral oil	11	192 ± 5	50 ± 0.9	5.30 ± 0.10	1.30 ± 0.06	0.55 ± 0.02	1.10 ± 0.12	15.4 ± 3.0	8.85 ± 0.54	43 ± 3
Saline + CCl_4	10	194 ± 3	51 ± 0.6	5.58 ± 0.06	1.11 ± 0.05	0.46 ± 0.02	0.87 ± 0.07	6.2 ± 0.6	34.0 ± 2.6	189 ± 12
Zymosan + CCl_4	10	195 ± 3	38 ± 0.5	8.29 ± 0.20	1.84 ± 0.06	2.06 ± 0.09	0.65 ± 0.06	11.2 ± 1.6	13.6 ± 1.6	113 ± 9

[a] Values are expressed as means ± standard error.

TABLE II

Influence of Carbon Tetrachloride on Plasma Bromsulphalein, and Phagocytic Function in Normal and RE Stimulated Rats [a]

Group	No. rats	Body weight, g	Organ weight, g			Hematocrit, %	Colloidal carbon, t/2	Plasma BSP, mg/ml
			Liver	Lung	Spleen			
Saline + mineral oil	6	223 ± 6	6.52 ± 0.39	1.61 ± 0.07	0.68 ± 0.03	46.2 ± 1.2	8.6 ± 1.2	0.47 ± 0.05
Saline + CCl_4	6	227 ± 9	7.20 ± 0.20	1.67 ± 0.11	0.61 ± 0.03	44.0 ± 3.0	8.3 ± 2.3	0.61 ± 0.03
Zymosan + mineral oil	5	226 ± 16	10.57 ± 0.45	2.94 ± 0.34	2.57 ± 0.19	32.8 ± 2.6	0.96 ± 0.20	0.31 ± 0.03
Zymosan + CCl_4	6	228 ± 11	10.47 ± 0.44	2.54 ± 0.09	2.18 ± 0.12	42.2 ± 3.2	1.10 ± 0.19	0.45 ± 0.02

[a] Values are means ± standard error.

lung, and spleen weights and a colloidal carbon half-time of approximately 1 min. In agreement with the findings in the saline group, carbon tetrachloride did not modify phagocytic function in the zymosan group.

The intravascular removal of BSP was significantly and similarly impaired in both the saline and zymosan carbon tetrachloride-treated groups, as the plasma BSP was increased approximately 40%.

The differences in plasma BSP concentration between the saline and zymosan groups reflects the increased plasma volume in the latter rats as blood volumes determined by the extrapolation of the colloidal carbon disappearance curve to zero-time were unaltered, while the hematocrits were significantly reduced in all but one group. The decreased plasma FFA concentration in the zymosan groups is also primarily due to the increased plasma volume.

Data confirming the impairment in BSP removal was obtained in an identical experiment with 24 additional rats where the BSP test for liver function was conducted without a prior injection of carbon. The mean 5 min plasma concentrations of BSP was 0.38 mg/ml in the saline-mineral oil group and 0.56 mg/ml in the saline-carbon tetrachloride group. Similarly, the mean concentration of plasma BSP was 0.28 mg/ml and 0.41 mg/ml in the zymosan mineral oil and carbon tetrachloride groups, respectively. A mean 20% reduction in hematocrit was also observed in both the zymosan groups.

Discussion

The development of a fatty liver resulting from acute carbon tetrachloride intoxication has been amply demonstrated. The mechanism by which carbon tetrachloride produces major derangements in triglyceride metabolism is not fully clarified but numerous theories have been proposed. The metabolic defects resulting in triglyceride elevation include such possibilities as: increased mobilization of fat from adipose tissue, increased hepatic lipogenesis, and depressed hepatic triglyceride catabolism. The most recent hypothesis is that carbon tetrachloride induces changes in the endoplasmic reticulum which impairs the hepatic triglyceride secretory mechanism, resulting in a failure in the transfer of triglycerides as lipoproteins from liver to plasma (14,15). The decreased plasma triglyceride level observed in present and past studies (14,15) appears to support this concept. Preliminary data has also been obtained in this laboratory which demonstrated a subsequent elevation in plasma triglyceride levels, which coincided with the reduction in the liver triglyceride concentration during recovery from acute carbon tetrachloride intoxication.

The present experiments contribute little to the understanding of the possible metabolic defects which lead to the accumulation of triglycerides in liver in carbon tetrachloride treated animals, or their modification in RE hyperfunctional rats. It is conceivable that the newly formed proliferated Kupffer cells which result after zymosan administration are much more resistant to the carbon tetrachloride injury. This possibility is suggested by the observations of Lacquet (16), who observed increased resistance of a newly regenerated liver to carbon tetrachloride.

It is also possible that rats with induced RE hyperplasia have an increased ability to metabolize the accumulated endogenous lipid, since the capability of RE cells to metabolize triglycerides has been demonstrated (17). It is also conceivable that if the concept of an impairment in the hepatic triglyceride secretory mechanism following carbon tetrachloride is valid, the failure of rats with induced Kupffer cell hyperplasia to develop a comparable fatty liver as observed in the control group may indicate that part of the secretory mechanism lies within the Kupffer cells which line the hepatic sinusoids and therefore are morphologically suitable for the location of a secretory mechanism. In this regard, employing retrograde intrabiliary injections of colloidal materials, the transfer of particles from the hepatic parenchymal cell to the Kupffer cell and discharge into plasma was demonstrated (17). Maintenance of the plasma triglyceride levels in zymosan-treated rats is suggestive of this possibility.

It is obvious, however, that a definitive mechanism regarding the significant inhibition of the fatty liver in RE stimulated rats following acute carbon tetrachloride exposure cannot now be proposed and must await further studies.

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Purification of Erucic Acid by Low-Temperature Crystallization

J. W. HAGEMANN, K. L. MIKOLAJCZAK, and I. A. WOLFF, Northern Regional Research Laboratory,[1] Peoria, Illinois

Abstract

Purification of erucic acid for laboratory use by low-temperature crystallization from aqueous acetone, ethanol, and methanol has been investigated. Two crystallizations of technical grade commercial acid (76–86%) from acetone-water (5:1) provided products of 94–98% purity, depending on the original composition; residual impurities were primarily C_{20} monoene and C_{22} saturated acids.

A CONVENIENT laboratory procedure was desired for purification of erucic acid for other chemical studies. Low-temperature crystallization procedures employing aqueous systems in conjunction with other methods have been described previously (1–4). Work has also been reported in which nonaqueous solvents (5–7) were employed. One of these (7) in our hands gave results inferior to the procedures described here, which use as crystallization solvents, acetone, ethanol, and methanol, with varying amounts of water. Evaluation of product purities by gas-liquid chromatography (GLC) (8) provided more adequate characterization than that given by most previous investigators.

Table I shows the composition of the starting materials. The mixed acids from *Crambe abyssinica* seed oil were chosen for investigating application of our crystallization techniques to a natural erucic acid source of lesser erucic acid content than the technical product. *Crambe abyssinica* is a heavy-seeding annual of the Cruciferae family, having good crop potential and widespread adaptability for growth in the U. S., and its oil has the highest erucic acid content of any cruciferous seed oil analyzed (9). Tables II, III, and IV show results of the crystallization experiments. In each case the same solvent combination and temperature conditions were used throughout a particular run.

GLC was used for purity evaluations; there was no significant difference in iodine value between erucic acid and the less pure products from the filtrates.

Of the three solvent systems, acetone-water was the most effective under the test conditions. That solvent gave the most efficient removal of C_{18} polyunsaturated acids from erucic in the purification of mixed acids from *Crambe abyssinica* oil. The most persistent impurities were eicosenoic, behenic, and oleic acids. Optimum purification of erucic acid from sources such as *Crambe* oil was not achieved by this procedure alone. However when a commercial concentrate (86%) is used as the raw material source, the method conveniently provides working quantities of purified erucic acid with adequate recovery.

[1] A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, USDA.

Experimental

Materials. Ground seeds of *Crambe abyssinica* (270 g, air-equilibrated basis) were extracted overnight in a Soxhlet apparatus with petroleum ether (30–60C). Most of the solvent was evaporated on a steam bath at atmospheric pressure, and the remainder was removed *in vacuo*. The oil (102.1 g) was then refluxed with 1 l of 0.8 *N* ethanolic KOH under nitrogen for 3 hr. Water was added to the soap mixture and it was then extracted with ethyl ether to remove unsaponifiable matter (6.27 g), acidified with dilute HCl, and the free fatty acids were recovered (95.1 g) and used for the purification experiments.

Two batches of an erucic acid concentrate used in these experiments (76 and 86% pure by GLC) were purchased from the Archer-Daniels-Midland Co.

Crystallization Procedure. The erucic acid (10 g) was first dissolved in the organic solvent (20 ml). Measured amounts of water and the organic solvent were then added alternately to this solution in such a way that the resultant mixture became clear after slight warming on a steam bath. Once the proper solvent ratios were found (Tables II, III, and IV), this procedure was not repeated until a different solvent ratio was desired. The mixture was then cooled immediately and held for 1 hr at the crystallization temperature being investigated. The crystals were rapidly filtered, without washing with solvent, onto Whatman No. 1 filter paper, in an unchilled Buchner funnel, and then placed in a vacuum desiccator to remove last traces of solvent and water. After determining the weight and withdrawing a sample for methyl ester preparation, the second crystallization was done using the same conditions as the first.

Methyl Esters. The methyl esters for GLC an-

TABLE I

Composition of Starting Material by Gas-Liquid Chromatography[a]

Chain length	Commercial erucic acid		*Crambe abyssinica* seed oil acids
	86%	76%	56%
$C_{12}S$[b]	Trace	....	....
$C_{14}S$	....	....	Trace
$C_{16}S$	0.2	0.2	2.4
$C_{16}I$	....	....	0.4
$C_{18}S$	0.2	0.6	0.5
$C_{18}I$	1.9	}	18.0
$C_{18}II$	1.6	} 9.1	9.0
$C_{18}III$	0.1	}	6.7
$C_{20}S$	0.4	1.8	0.7
$C_{20}I$	7.2	9.9	4.2
$C_{20}II$	Trace	....	....
$C_{22}S$	1.8	2.9	0.8
$C_{22}I$	86.0	76.0	56.0
$C_{22}II$	0.2	....	....
$C_{24}I$	0.5	....	0.3
Other	....	....	0.3

[a] Area percentage of methyl ester peaks.
[b] I denotes the monoene; II, the diene; III, the triene; and S, the saturated fatty acids.