Effects of Trichloroethylene, 1,1,1-Trichloroethane and Carbon Tetrachloride on Plasma Lipoproteins of Rats

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(Received July 17, 1990 and in revised form August 27, 1990)

Abstract: Effects of single intraperitoneal administration of trichloroethylene, 1,1,1-trichloroethane, and carbon tetrachloride (positive control) on the plasma contents of lipoproteins were investigated in rats. Plasma was fractionated to VLDL, LDL, and HDL by sequential ultracentrifugation. On the administration of carbon tetrachloride at 30 to 1000 mg/kg, VLDL and HDL were reduced dose-dependently, but the reduction in LDL was not dose-dependent. With trichloroethylene at 30 to 300 mg/kg, the lipid contents of VLDL and LDL fractions were decreased. At 1000 mg/kg, VLDL and LDL were increased by the trichloroethylene. The HDL was decreased with increasing doses of trichloroethylene at 30 to 1000 mg/kg. With 1,1,1-trichloroethane at 100 to 300 mg/kg, VLDL and LDL were increased. The HDL levels rose at 100 mg/kg but fell at 1000 mg/kg. Thus trichloroethylene impairs VLDL formation at low doses. 1,1,1-Trichloroethane stimulates the VLDL synthesis at low doses and inhibits it at high doses. The decreases in HDL at high doses of trichloroethylene and 1,1,1-trichloroethane resulted from the inhibition of HDL synthesis. Liver-to-body weight ratios were raised with increasing doses of carbon tetrachloride, trichloroethylene, and 1,1,1-trichloroethane. Plasma GOT and GPT activities rose at much higher doses of solvents than dose levels which produce the changes in lipoproteins and the increases in liver weights. The liver enlargement appeared to be a sensitive marker of hepatotoxicity related to the changes in lipoproteins, the profile of which was different in three solvents.

Key words: Trichloroethylene—1,1,1-Trichloroethane—Carbon tetrachloride—Plasma—Lipoproteins—VLDL—LDL—HDL—Hepatotoxicity—GOT—GPT—Rat

INTRODUCTION

Trichloroethylene and 1,1,1-trichloroethane are widely used in indus-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; VHDL, very high density lipoproteins; d, density; EDTA, ethylenediamine-tetraacetic acid; DTNB, 5,5'-dithiobis (2nitrobenzoic acid); GOT, Glutamic oxaloacetic transaminase; GPT, Glutamic pyruvic transaminase.

try, mostly as a cleaning solvent. In humans, many chlorinated hydrocarbon solvents including trichloroethylene cause liver and kidney injury and some dysfunctions of the central nervous system.^{1,2)} Cardiac sensitization has also been reported by 1,1,1-trichloroethane exposure.^{3,4)} In experimental animals trichloroethylene and 1,1,1-trichloroethane cause central nervous system depression.⁵⁻⁷⁾ Hepatotoxicity has been reported also in rats and mice on exposure to high doses of trichloroethylene and 1,1,1-trichloroethane.^{8,9)} With exposure to some chlorinated hydrocarbon solvents which are relatively toxic, such as carbon tetrachloride, the concentrations of plasma lipoproteins decrease.¹⁰⁾ Apparently this results from a decrease in the synthesis and secretion of the lipoproteins. These changes in lipoprotein metabolism appear to be due to the liver injury produced by the hepatotoxins.¹¹⁾

In this study the effects of trichloroethylene and 1,1,1-trichloroethane on plasma lipoproteins of rats were investigated in relation to the changes in liver and plasma transaminase activities. These effects were compared with carbon tetrachloride effects (positive control) as preliminaries for the evaluation of hepatotoxicity of chlorinated hydrocarbon compounds, since the toxicity of these compounds is not sufficiently clarified. We also hoped that lipoproteins might be more sensitive markers of hepatotoxicity since plasma transaminase activities, which are widely used as markers of hepatotoxicity, are neither sensitive enough nor always specific to liver injury.

MATERIAL AND METHODS

Animals

We used male Fischer 344 rats, 5- to 6-month-old (Japan Charles River) supplied chow diet (CE-2, Nihon Clea, Japan) and tap water ad libitum.

Organic Solvent Administration

Organic solvents were dissolved in 1 ml of olive oil/kg body weight, and injected intraperitoneally at 19 hr before sacrifice. Solvents used were carbon tetrachloride (specially prepared for spectroscopy, Nacalai tesqu Inc., Japan), trichloroethylene (Dotite Spectrosol[®], Dojindo Laboratories, Japan), and 1,1,1-trichloroethane (specially prepared for electronics, Nacalai tesqu Inc.)

Lipoprotein Preparation

The rats were fasted overnight before they were killed. Following anesthesia with diethylether, the rats were exsanguinated via the abdominal vein. Blood was collected on ice in tubes containing 1 mg of EDTA/ml, and plasmas were separated by centrifugation. Each plasma sample was added 0.1 mg of sodium azide/ml, and 0.01 mg of DTNB/ml.

The various density fractions of plasma lipoproteins were obtained by prepara-

tive ultracentrifugation in a RP-80T rotor (Hitachi, Japan) at 49,800 rpm in SCP-70H ultracentrifuge (Hitachi) at 10°C. The density of the plasma samples was adjusted by adding NaBr solutions containing 0.15 M NaCl, 0.1% EDTA, 0.01% Na azide, and 0.001% DTNB. The plasmas were pipetted into 5.4-ml ultracentrifuge tubes. The tubes were filled with a solution of NaCl at d 1.006 containing EDTA, Na azide, and DTNB. These were centrifuged for 16 hr. The top 1.5 ml, containing the VLDL fraction, was carefully removed. The density in the tube was adjusted to 1.063. After centrifuging for 22 hr, the LDL fraction in the top 1.5 ml was removed. To isolate the HDL fraction the density was raised to 1.210, and the top 1.5 ml was removed after centrifuging for 40 hr. The second 3.0 ml was removed as medium fraction, and the residual 0.9 ml was removed as the bottom fraction.

Chemical Analysis

The plasma and each lipoprotein fraction were analyzed for lipids by enzymatic procedures. Free and total cholesterol were analyzed using Free cholesterol[®] and Monotest cholesterol[®] kits (Boeringer-mannheim), respectively. Esterified cholesterol was calculated from the difference of total and free cholesterol determinations. Determiner TG-S555 (Kyowa Medex, Japan) and PL Kainos (Kainos Laboratories, Japan) kits were used to analyze triglyceride and phospholipid, respectively. The protein contents of the plasma and each lipoprotein fraction were estimated by a modified Lowry method.¹²⁾

Statistical Analysis

Differences among the four groups were analyzed by analysis of variance (F-test). Dunnett's multiple *t*-test was also used to compare the control and the solvent-treated groups. A *p*-value of <0.05 was chosen to indicate significance.

RESULTS

Conditions for Ultracentrifugation

To obtain the appropriate time for the separation of rat lipoproteins, the plasmas were divided to 2.2 ml fraction and were centrifuged at d 1.006 for various hours. The top 1.5 ml fractions were removed as VLDL fractions. Other fractions of rat plasma were centrifuged for 22 hr at d 1.006 and the VLDL fractions were removed. By adding NaBr solution, the density in the tubes was raised to 1.063, and these tubes were centrifuged for various hours. The top 1.5 ml fractions were removed as LDL fractions. The VLDL and LDL fractions of other groups of plasma were removed by centrifugation at d 1.006 for 22 hr and at 1.063 for 22 hr, respectively. Then the density in the tubes was adjusted to 1.125. Following centrifugation for 23–46 hr, the top 1.5 ml fractions were removed as HDL₂. From other groups of plasma fractions, VLDL, LDL,

Fig. 1. The concentrations of cholesterol, triglyceride, phospholipid, and protein in the top fractions of plasma after ultracentrifuging for various hours at different densities. Milligrams in 100 ml of plasma.

●; Protein □; Phospholipid.
○; Cholesterol. △; Triglyceride.

and HDL₂ fractions were removed (46-hr centrifugation for floating HDL₂), and the remaining fractions were centrifuged for 23–46 hr at d 1.210. The top 1.5 ml was removed as HDL₃. The lipid and protein contents of these fractions were determined.

Figure 1 shows the concentrations of total cholesterol, triglyceride, phospholipid, and protein in each density fraction after centrifugation for different hours. Concentrations are expressed in terms of 100 ml of the plasma from which the lipoprotein fractions had been separated. At d 1.006, the triglyceride, phospholipid, and protein reached a minimum at the end of 16-hr centrifugation. At d 1.063, the concentrations of the three lipids were minimal following 12 hr of centrifugation, and the protein concentration reached minimum after 16-hr centrifugation. At d 1.125, the triglyceride concentration remained constant after 23 and 46 hr of centrifugation. Cholesterol and phospholipid concentrations were gradually elevated by increasing the hours of centrifugation and reached a maximum at 42 hr. Protein concentration reached a minimum at the end of 39 hr of centrifugation. At d 1.210, the concentrations of cholesterol, phospholipid,

and protein was constant after 39 hr or more of centrifugation.

These results showed that the minimum centrifugation period for complete flotation of VLDL at $d\ 1.006$ and LDL at $d\ 1.063$ was 16 hr, and that of HDL at $d\ 1.210$ was 39 hr. Therefore, the separations of rat plasma after solvent administrations were carried out by sequential ultracentrifugations for 16 hr for VLDL, 22 hr for LDL, and 40 hr for HDL.

Level of Plasma Lipoproteins

Several time course studies showed that the maximal changes in serum GPT activity and serum triglyceride content occurred between 16 and 34 hr following the injection of 30 to 1000 mg of carbon tetrachloride/kg. Maximal changes in these parameters induced by 1000 mg trichloroethylene/kg occurred between 4 and 24 hr. With 1000 mg 1,1,1-trichloroethane/kg, maximal changes were produced between 4 and 19 hr following the injection. From these preliminary data, 19 hr was chosen as an interval between the administration of organic solvents and the sacrifice of the rats.

In a first series of experiments, the level of plasma lipoproteins was determined in control rats and in rats treated with 30, 100, and 1000 mg of carbontetrachloride per kg of body weight. The lipids and protein composition of the unfractionated plasma, and the medium and bottom fractions at $d\ 1.210$ were also determined. Tables 1A and 1B summarize the results.

Cholesterol, triglyceride, phospholipid, and protein concentrations in the plasma and lipoprotein fraction were significantly lower in carbon tetrachloride-treated rats at 30 mg/kg than in control rats except the protein contents of unfractionated plasma and medium fraction, and the triglyceride contents of HDL, medium and bottom fractions. This effect of carbon tetrachloride was dose-dependent in the plasma, VLDL, HDL, medium, and bottom fractions. Decreases in the lipid contents of LDL fraction were maximal at 30 or 100 mg/kg.

Tables 2A and 2B show the effects of single administration of 30, 300, and 1000 mg of trichloroethylene/kg. The lipid contents of the VLDL and LDL fractions were lower in the 30 and 300 mg/kg groups than in the control group. However, those in the 1000 mg/kg group were higher than in the control group. In HDL fraction lipid concentrations were decreased dose dependently by the administartion of trichloroethylene but triglyceride was not. Protein concentrations in the VLDL and LDL fractions were increased and those in the HDL fraction were decreased by the trichloroethylene.

The effects of administration of 1,1,1-trichloroethane at 100, 300, and 1000 mg/kg are summarized in Tables 3A and 3B. The lipid and protein levels in VLDL and LDL fractions increased on 1,1,1-trichloroethane. The maximal increase in the VLDL and LDL fractions occurred at 300 and 100 mg/kg, respectively. Lipid and protein levels in the HDL fraction increased at 100 mg/kg and decreased at 1000 mg/kg.

Table 1A. Effects of single administration of carbon tetrachloride on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
Plasma								
Control	8.8±0.5	41.4±1.6	50.3±1.9	65.9±0.9	103.3±3.6	219.5±5.0	3765±127	
30 mg/kg	4.4±0.2**	22.5±0.7**	26.9±1.0**	39.6±0.9**	62.3±2.2**	129.0±3.4**	3830±22	
100 mg/kg	3.0±0.1**	18.3±0.4**	21.3±0.4**	31.9±0.5**	56.1±0.8**	109.3±1.1**	3885±76	
1000 mg/kg	2.9±0.3**	10.8±0.6**	13.7±0.8**	33.7±0.5**	50.0±1.1**	97.4±2.1**	3831±50	
F-test	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	NS	
VLDL								
Control	0.61±0.05	0.55±0.02	1.16±0.07	25.97±2.30	4.36±0.42	31.49±2.76	2.30±0.25	33.80±2.51
30 mg/kg	0.07±0.04**	0.21±0.01**	0.28±0.04**	10.03±0.23**	0.54±0.07**	10.85±0.28**	1.03±0.16**	11.87±0.38**
100 mg/kg	0.04±0.04**	0.23±0.01**	0.27±0.04**	7.73±0.48**	0.21±0.12**	8.20±0.58**	0.70±0.03**	8.90±0.56**
1000 mg/kg	0.03±0.03**	0.21±0.02**	0.24±0.04**	5.03±0.12**	0.12±0.07**	5.38±0.19**	0.43±0.15**	5.82±0.24**
F-test	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p
LDL								
Control	2.92±0.27	4.75±0.48	7.67±0.74	9.40±0.50	10.53±1.04	27.6±2.2	4.35±0.07	32.0±2.2
30 mg/kg	1.54±0.03*	2.41±0.05**	3.96±0.07**	6.26±0.31**	4.91±0.08**	15.1±0.33**	2.74±0.33**	17.9±0.6**
100 mg/kg	1.75±0.06*	2.38±0.10**	4.13±0.14**	6.78±0.31**	4.83±0.30**	15.7±0.7**	2.18±0.13**	17.9±0.8**
1000 mg/kg	3.23±0.54	1.26±0.02**	4.54±0.52**	8.00±0.71	8.90±1.42	21.4±2.6*	1.86±0.18**	23.3±2.6**
F-test	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p

FC, free cholesterol; EC, esterified cholesterol; TC, total cholesterol; TG, triglyceride; PL, phospholipid; TL, total lipid; PR, protein. Milligrams per fraction in 100 ml of plasma. Each value represents the mean $\pm$ SEM (N=5).

* and ** represent significant differences at 0.05>p and 0.01>p, respectively, between control and solvent-treated groups by Dunnett's test. NS, 0.05>p, and 0.01>p in the table represent not significant, significant at 0.05>p, and significant at 0.01>p, respectively, by the analysis of variance (F-test).

Table 1B. Effects of single administration of carbon tetrachloride on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
HDL								
Control	4.89±0.04	22.53±0.39	27.42±0.43	3.33±0.12	44.2±0.5	74.9±0.9	42.0±1.6	117.0±2.1
30 mg/kg	3.27±0.09**	12.94±0.52**	16.20±0.61**	3.16±0.21	26.2±0.8**	42.6±3.8**	30.7±1.6**	73.3±3.1**
100 mg/kg	2.95±0.08**	10.19±0.30**	13.14±0.37**	2.66±0.09*	24.0±0.7**	39.8±1.0**	22.8±2.2**	62.7±3.2**
1000 mg/kg	3.00±0.15**	3.90±0.76**	6.90±0.68**	3.19±0.25	16.1±2.3**	26.2±2.8**	12.4±2.5**	38.6±5.3**
F-test	0.01>p	0.01>p	0.01>p	NS	0.01>p	0.01>p	0.01>p	0.01>p
MF								
Control	0	4.53±0.42	4.53±0.42	6.95±0.33	11.14±0.52	22.6±0.9	149±6	
30 mg/kg	0	2.41±0.06**	2.41±0.06**	6.67±0.77	5.31±0.18**	14.4±0.7**	144±13	
100 mg/kg	0	1.65±0.08**	1.65±0.08**	7.00±0.51	3.88±0.31**	12.5±0.8**	176±15	
1000 mg/kg	0	0.84±0.05**	0.84±0.05**	8.18±0.82	2.87±0.18**	11.9±0.7**	160±10	
F-test	—	0.01>p	0.01>p	NS	0.01>p	0.01>p	NS	
BF								
Control	0	1.09±0.21	1.09±0.21	2.84±0.11	22.4±1.0	26.4±1.2		
30 mg/kg	0	0.71±0.03*	0.71±0.03*	2.58±0.13	16.4±0.7**	19.7±0.7**		
100 mg/kg	0	0.66±0.04*	0.66±0.04*	2.83±0.07	16.0±0.4**	19.5±0.6**		
1000 mg/kg	0	0.69±0.03*	0.69±0.03*	3.50±0.38	10.1±0.5**	14.3±0.4**		
F-test	—	0.05>p	0.05>p	0.05>p	0.01>p	0.01>p		

Legends are as in Table 1A.

Table 2A. Effects of single administration of trichloroethylene on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
Plasma								
Control	7.18±0.13	47.5±1.0	54.7±1.1	51.2±0.7	83.0±1.7	189±3	4289±210	
30 mg/kg	5.82±0.15**	43.8±1.3	49.6±1.4	52.9±2.4	81.1±1.2	184±5	4235±104	
300 mg/kg	5.71±0.25**	41.0±1.7**	46.7±2.0**	53.6±0.8	72.8±2.6**	173±5	4324±81	
1000 mg/kg	7.06±0.22	38.2±1.3**	45.3±1.5**	66.1±2.8**	71.8±2.2**	183±6	3908±85	
F-test	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	NS	NS	
VLDL								
Control	0.84±0.04	0.75±0.04	1.59±0.08	21.5±0.8	3.32±0.19	26.4±1.0	1.59±0.06	28.0±1.0
30 mg/kg	0.70±0.05	0.76±0.04	1.47±0.04	19.1±0.8	2.86±0.09	23.4±0.9	1.85±0.07*	25.3±0.9
300 mg/kg	0.55±0.19**	0.76±0.01	1.31±0.04*	18.2±0.2	2.46±0.17**	22.0±0.3*	1.81±0.05	23.8±0.4
1000 mg/kg	1.02±0.17*	0.77±0.03	1.79±0.08	26.7±1.9**	3.93±0.23*	32.5±2.2**	2.38±0.08**	34.8±2.2**
F-test	0.01>p	NS	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p
LDL								
Control	4.27±0.10	9.10±0.44	13.4±0.5	9.91±0.29	15.2±0.8	38.5±1.2	6.44±0.15	44.9±1.4
30 mg/kg	3.46±0.02**	7.62±0.21	11.1±0.2	10.49±0.33	13.2±0.3	34.8±0.4	6.24±0.15	41.0±0.4
300 mg/kg	3.42±0.14**	7.50±0.83	10.9±1.0	10.49±0.35	12.2±1.2	33.6±2.5	6.62±0.09	40.2±2.6
1000 mg/kg	5.05±0.21**	9.74±0.71	14.8±0.9	12.68±0.75**	17.0±1.1	44.5±2.4	6.83±0.50	51.4±2.8
F-test	0.01>p	0.05>p	0.01>p	0.01>p	0.05>p	0.01>p	NS	0.01>p

Legends are as in Table 1A.

Table 2B. Effects of single administration of trichloroethylene on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
HDL								
Control	5.88±0.08	30.6±0.7	36.4±0.8	2.93±0.08	46.4±1.1	85.8±1.8	45.8±3.2	132±5
30 mg/kg	4.98±0.21**	28.5±0.7	33.5±0.8	3.30±0.09	44.6±1.0	81.3±1.9	38.9±1.1	120±3
300 mg/kg	4.72±0.19**	25.0±1.8**	29.7±1.9**	4.12±0.10**	37.9±2.6**	71.7±4.4**	34.6±3.4	106±8*
1000 mg/kg	5.22±0.13*	21.1±0.5**	26.3±0.5**	3.76±0.16**	39.1±0.5**	69.2±1.2**	37.8±4.6	107±6*
F-test	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	0.01>p	NS	0.05>p
MF								
Control	0	5.35±0.10	5.35±0.10	6.25±0.06	11.04±0.76	22.7±0.8	180±9	
30 mg/kg	0	5.64±0.19	5.64±0.19	7.31±0.06	13.33±0.91	26.3±1.1*	219±4	
300 mg/kg	0	5.25±0.46	5.25±0.46	10.46±0.69**	10.43±0.47	26.1±0.8*	230±19	
1000 mg/kg	0	4.93±0.29	4.93±0.29	8.72±0.51**	8.41±0.46*	22.1±0.7	226±21	
F-test	—	NS	NS	0.01>p	0.01>p	0.01>p	NS	
BF								
Control	0	1.17±0.08	1.17±0.08	2.48±0.08	25.6±0.4	29.5±0.5		
30 mg/kg	0	1.08±0.07	1.08±0.07	3.00±0.17	26.2±0.5	30.2±0.5		
300 mg/kg	0	1.04±0.07	1.04±0.07	4.40±0.30**	23.2±0.6*	28.6±0.7		
1000 mg/kg	0	1.04±0.08	1.04±0.08	3.59±0.05**	18.5±0.8**	22.9±0.7*		
F-test	—	NS	NS	0.01>p	0.01>p	0.01>p		

Legends are as in Table 1A.

Table 3A. Effects of single administration of 1, 1, 1-trichloroethane on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
Plasma								
Control	8.60±0.25	45.8±1.1	54.4±1.2	65.9±1.5	89.2±1.6	210±3	3769±101	
100 mg/kg	10.48±1.05	46.8±1.2	57.3±1.9	61.8±4.2	93.8±2.3	213±7	3662±128	
300 mg/kg	7.59±0.42	48.9±1.0	56.5±1.4	47.0±3.5**	94.9±2.0	198±7	3981±64	
1000 mg/kg	6.82±0.26	44.8±1.3	51.6±1.5	46.6±1.1**	90.2±0.8	188±3*	4005±52	
F-test	0.01>p	NS	NS	0.01>p	NS	0.05>p	0.05>p	
VLDL								
Control	0.52±0.03	0.61±0.08	1.14±0.09	18.2±1.5	2.79±0.06	22.2±1.5	2.38±0.06	24.5±1.4
100 mg/kg	0.39±0.04*	0.49±0.14	0.85±0.17	24.5±1.9*	2.39±0.26	27.7±2.3	1.95±0.07	29.7±2.4
300 mg/kg	0.69±0.05**	0.46±0.07	1.15±0.11	31.0±1.5**	2.87±0.13	35.0±1.7**	3.20±0.19**	38.2±1.9**
1000 mg/kg	0.49±0.03	0.27±0.02*	0.76±0.04	24.1±1.4*	2.64±0.13	27.6±1.5	2.69±0.19	30.2±1.7
F-test	0.01>p	NS	NS	0.01>p	NS	0.01>p	0.01>p	0.01>p
LDL								
Control	3.88±0.35	8.67±0.17	12.6±0.5	15.1±0.3	15.5±0.8	43.1±1.1	10.80±0.13	53.9±1.2
100 mg/kg	7.42±0.67**	13.99±0.98**	21.4±1.6**	17.2±2.1	25.7±2.1**	64.3±5.7**	13.39±1.08*	77.8±6.8**
300 mg/kg	4.16±0.22	12.10±0.59**	16.3±0.8*	16.8±0.9	20.0±0.8	53.0±1.5	11.52±0.35	64.5±1.7
1000 mg/kg	2.81±0.28	7.50±0.69	10.3±1.0	16.3±1.9	13.4±1.3	40.0±4.1	7.93±0.62*	48.0±4.7
F-test	0.01>p	0.01>p	0.01>p	NS	0.01>p	0.01>p	0.01>p	0.01>p

Legends are as in Table 1A.

Table 3B. Effects of single administration of 1, 1, 1-trichloroethane on the plasma lipoproteins of rats

	FC	EC	TC	TG	PL	TL	PR	TL+PR
HDL								
Control	5.87±0.21	33.9±0.5	39.7±0.7	4.04±0.21	48.6±1.3	92.3±1.8	43.8±0.6	136±2
100 mg/kg	7.79±0.51**	36.8±0.9	44.5±1.4	4.90±0.33	57.8±3.0*	107.2±4.6*	49.9±2.1*	157±7*
300 mg/kg	6.24±0.28	35.5±0.9	41.7±1.2	4.45±0.17	52.5±1.1	98.6±2.1	46.8±0.2	145±2
1000 mg/kg	4.98±0.56	21.3±1.7**	26.1±2.2**	3.50±0.65	36.2±3.4**	65.8±6.0**	35.3±2.3**	101±8**
F-test	0.01>p	0.01>p	0.01>p	NS	0.01>p	0.01>p	0.01>p	0.01>p
MF								
Control	0	3.17±0.25	3.17±0.25	7.84±0.20	10.48±0.46	21.5±0.5	166±2	
100 mg/kg	0	3.45±0.23	3.45±0.23	11.36±1.57	11.94±1.50	25.6±2.4	173±12	
300 mg/kg	0	4.45±0.34*	4.45±0.34*	12.03±2.79	11.89±0.35	25.1±0.6	152±2	
1000 mg/kg	0	2.26±0.42	2.26±0.42	7.71±1.43	7.48±0.99	16.7±2.7	105±11**	
F-test	—	0.01>p	0.01>p	NS	0.05>p	0.05>p	0.01>p	
BF								
Control	0	1.53±0.33	1.53±0.33	2.31±0.12	26.3±0.4	30.2±0.6		
300 mg/kg	0	0.94±0.09	0.94±0.09	1.93±0.40	25.6±1.2	28.5±1.6		
300 mg/kg	0	0.91±0.05*	0.91±0.05*	0.16±0.07**	24.2±0.4	25.2±0.4*		
1000 mg/kg	0	0.75±0.07*	0.75±0.07*	0.08±0.05**	17.9±1.3**	18.8±1.4**		
F-test	—	0.05>p	0.05>p	0.01>p	0.01>p	0.01>p		

Legends are as in Table 1A.

Table 4. Effects of single administration of solvents on the esterified-to-free cholesterol ratios of rat lipoprotein fractions

CTC	VLDL	LDL	HDL	TCen	VLDL	LDL	HDL	TCen	VLDL	LDL	HDL
Control	0.90	1.63	4.61	Control	0.89	2.13	5.20	Control	1.17	2.23	5.78
30 mg/kg	3.00	1.56	3.96	30 mg/kg	1.09	2.20	5.72	100 mg/kg	1.26	1.89	4.72
100 mg/kg	5.75	1.36	3.45	300 mg/kg	1.38	2.19	5.30	300 mg/kg	0.67	2.91	5.69
1000 mg/kg	7.00	0.39	1.30	1000 mg/kg	0.75	1.93	4.04	1000 mg/kg	0.55	2.67	4.28

The ratios were calculated from the mean values of esterified and free cholesterol contents of each lipoprotein fraction.
 CTC, carbon tetrachloride; TCen, trichloroethylene; TCen, 1,1,1-trichloroethane.

Table 5. Effects of administration of carbon tetrachloride, trichloroethylene, and 1,1,1-trichloroethane on the body weight, liver weight, and liver-to-body weight ratio of rats.

Solvent	Dose	Body Weight (g)	Liver Weight (g)	Liver/Body Weight Ratio (%)
CTC	Control	356±3	8.30±0.11	2.33±0.01
	30 mg/kg	368±3	9.36±0.07**	2.55±0.02**
	100 mg/kg	360±2	10.18±0.17**	2.83±0.06**
	1000 mg/kg	364±5	11.04±0.39**	3.03±0.08**
	F-test	NS	0.01>p	0.01>p
TCen	Control	375±5	8.61±0.08	2.29±0.01
	30 mg/kg	382±7	9.09±0.21	2.38±0.02*
	300 mg/kg	369±4	8.90±0.10	2.41±0.02**
	1000 mg/kg	367±5	9.47±0.21**	2.58±0.03**
	F-test	NS	0.05>p	0.01>p
TCan	Control	395±9	9.33±0.23	2.36±0.01
	100 mg/kg	387±9	9.51±0.53	2.45±0.09
	300 mg/kg	382±8	9.23±0.23	2.42±0.02
	1000 mg/kg	374±8	9.50±0.28	2.54±0.05*
	F-test	NS	NS	NS

Liver-to-body weight ratios were calculated for each animal. See legends for Tables 1A and 4.

Table 6. Effects of single administration of carbon tetrachloride, trichloroethylene, and 1,1,1-trichloroethane on plasma GOT and GPT activity of rats

CTC			TCen			TCan		
	GOT	GPT		GOT	GPT		GOT	GPT
Control	76±5	44±2	Control	84±7	50±4	Control	71±4	49±3
30 mg/kg	84±3	48±1	30 mg/kg	105±11	67±6	100 mg/kg	76±4	50±1
100 mg/kg	118±7	102±6	300 mg/kg	89±6	56±3	300 mg/kg	74±5	50±2
1000 mg/kg	1029±187**	548±88**	1000 mg/kg	197±8**	94±4**	1000 mg/kg	93±13	55±3
F-test	0.01>p	0.01>p	F-test	0.01>p	0.01>p	F-test	NS	NS

See legends for Tables 1A and 4.

The esterified-to-free cholesterol ratios of lipoprotein fractions were calculated in each group and are summarized in Table 4. Great changes in these ratios occurred in the carbon tetrachloride groups. The ratios were increased in the VLDL fraction but decreased in the LDL and HDL fractions.

Changes in Liver Weights

The bodies of the rats treated with organic solvents were weighed before exsanguination. The livers were weighed after exsanguination (Table 5). Liver weights and liver-to-body weight ratios were increased in rats treated with carbon tetrachloride at doses of 30 mg/kg or more. On the administration of trichloroethylene, statistically significant increase in liver weight occurred at 1000

mg/kg, and significant increase in liver-to-body weight ratio occurred at 30 mg/kg or more. 1,1,1-Trichloroethane at 1000 mg/kg produced a significant increase in liver-to-body weight ratio.

Increases in Plasma Transaminase Activities

Plasma transaminase activities in rats treated with organic solvents are shown in Table 6. Carbon tetrachloride had no effect on transaminase activities at 30 mg/kg; it elevated these enzyme activities at 100 mg/kg. However, these changes at 100 mg/kg were not statistically significant. A large, significant increase occurred at 1000 mg/kg. With trichloroethylene, a significant increase in GOT and GPT activities was observed at 1000 mg/kg. No effects were observed at 300 mg/kg. 1,1,1-Trichloroethane elicited no changes in transaminase activities except a slight but not significant increase in GOT at 1000 mg/kg.

DISCUSSION

We have described the changes in lipoproteins produced by a single administration of trichloroethylene and 1,1,1-trichloroethane.^{13,14)} We have found no such effects previously.

It is believed that the reduction in plasma lipoprotein levels by the administration of carbon tetrachloride is due to the liver injury followed by the inhibition of the synthesis and secretion of lipoproteins.¹¹⁾ In our study the components of plasma lipoproteins of rats injected with carbon tetrachloride were decreased dose dependently except the LDL fraction. The cholesterol, triglyceride, and phospholipid contents of the LDL fraction at 1000 mg carbon tetrachloride/kg was higher than those at 30 and 100 mg/kg. However, the protein content of LDL fraction at 1000 mg/kg was lower than at 30 or 100 mg/kg. Consequently, the lipid/protein ratio of LDL was elevated by high doses of carbon tetrachloride. This means the lipid/protein composition of the LDL at 1000 mg/kg was greatly different from the control, 30, and 100 mg/kg groups. The effect of high doses of carbon tetrachloride on LDL found in this experiment have not been previously reported. Plasma VLDL is synthesized in the liver and secreted into the blood stream, and is converted to LDL in the blood.¹⁵⁾ In the present experiment the conversion from VLDL to LDL appears to have been altered by the administration of 1000 mg carbon tetrachloride/kg in addition to the inhibition of VLDL synthesis which caused the decrease in VLDL and LDL contents at lower doses. Almost all lipid and protein components of the HDL, medium, and bottom fractions were reduced by carbon tetrachloride. The only exception was triglyceride. The synthesis of HDL and VHDL (very high density lipoproteins which are $d > 1.21$ lipoproteins and are included in the medium and bottom fractions) was lowered by carbon tetrachloride, as was VLDL synthesis. However, the lack of effects on triglyceride levels were not explained by our studies.

The decrease in the protein level of the medium fraction, which was expected to occur accompanying the reduction in VHDL, did not appear because the medium fraction was contaminated with a large amount of plasma proteins such as albumin.

In rats injected with 30 or 300 mg trichloroethylene/kg, the lipid contents of the VLDL and LDL fractions were decreased while the protein contents were unaltered. The impairment in the livers of the VLDL formation from the lipids and proteins could account for these phenomena. At 1000 mg/kg, both the inhibition of LDL uptake into the liver and the reduced velocity of conversion from VLDL to LDL apparently contribute to the simultaneous increases in VLDL and LDL. The plasma content of HDL decreased dose-dependently at 30 to 1000 mg/kg and this apparently resulted from the inhibition of HDL synthesis.

With 1,1,1-trichloroethane administration at 100 and 300 mg/kg, VLDL, LDL, and HDL were increased. Apparently the synthesis and excretion of these lipoproteins were stimulated by the 1,1,1-trichloroethane. However, the mechanism of these changes remains murky. HDL was reduced at 1000 mg/kg, possibly due to the inhibition of HDL synthesis.

In rats treated with carbon tetrachloride, the esterified/free cholesterol ratios were greatly increased in the VLDL and decreased in the LDL and HDL fractions. In the metabolic pathways of the cholesterol of rat lipoproteins, the esterified form of cholesterol is converted to free form by the cholesterol esterase while the free form is converted to the esterified form by the acyl-CoA cholesterol acyltransferase. These conversion processes appear to have been modified by carbon tetrachloride, particularly at high doses.

The increases in liver/body weight ratios by carbon tetrachloride and trichloroethylene were statistically significant at 30 mg/kg, and those by 1,1,1-trichloroethane were significant at 1000 mg/kg. The liver was enlarged by a single administration of the other chlorinated hydrocarbon compounds which have very weak hepatotoxicity (unpublished observation). This increase in liver weight induced by trichloroethylene was considered a toxic effect, although the response was attributed to hypertrophy of hepatocytes rather than regenerative hyperplasia, on the basis of histological examination.¹⁶⁾ The degree of enlargement was greatest with carbon tetrachloride administration while the least effect was observed with 1,1,1-trichloroethane. This order of potency was consistent with the degree of hepatotoxicity of these three compounds. No statistically significant increases occurred in either GOT or GPT (traditional markers of hepatotoxicity) at 100 mg carbon tetrachloride/kg, 300 mg trichloroethylene/kg, or 1000 mg 1,1,1-trichloroethane/kg. The liver enlargement appears to be a sensitive marker in the evaluation of hepatotoxicity of chlorinated hydrocarbon compounds.

The serum transaminase activities (GOT and GPT) have been believed to be the most sensitive markers of severity of hepatic injuries. In our study, the

effects of carbon tetrachloride on lipoprotein contents definitely appeared at 30 mg/kg. However, GOT activities were not altered at this dose. And the slight increases in these enzyme activities at 100 mg/kg were not statistically significant. With trichloroethane administration at 30 mg/kg, VLDL-protein was increased and LDL- and HDL-free cholesterol were decreased. At 300 mg/kg, significant changes occurred in a variety of determinations (VLDL-free cholesterol etc.). However, there were no changes in transaminase activities at these doses. 1,1,1-Trichloroethane elicited increases or decreases in many lipoprotein components at 100 mg/kg, but the GOT and GPT remained unchanged even at 1000 mg/kg. This indicates that the changes in lipoprotein and its components are much more sensitive markers for the effects of chlorinated hydrocarbon solvents. These changes are probably related to the hepatotoxicity of solvents as shown by the liver enlargement.

With carbon tetrachloride administration, the changes in the lipid components of fractionated lipoproteins were much greater than those of unfractionated plasma. With trichloroethylene, no significant changes were observed in the total lipid contents of plasma while those in VLDL were reduced at 300 mg/kg. With 1,1,1-trichloroethane, there were no significant changes in the lipid components of unfractionated plasma at 100 mg/kg but there were many changes in the VLDL, LDL, and HDL fractions at this dose. These results show that the fractionation of plasma into lipoproteins has advantages because the changes in lipoproteins are detectable at lower doses than the unfractionated plasma.

In this paper we describe the change in lipoproteins induced by a single administration of trichloroethylene and 1,1,1-trichloroethane. For the mechanism of these changes, some explanations are possible but the precise mechanism remains to be clarified. Such a study is now in progress.

ACKNOWLEDGMENTS

The author thanks Dr. Mitsuo Sato for his advice. The technical assistance of Mrs. Megumi Kanada (Suda) and Mr. Toshiki Yamanouchi is greatly appreciated.

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