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Research Article

Dose and Time Dependent Effects of Intraperitoneal Administration of Carbon Tetrachloride (CCl4) on Blood Lipid Profile in Wistar Rats

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Abstract

Marked alteration in the physiological concentrations of blood lipids has been implicated in the incidence of cardiovascular diseases and other metabolic disorders. Such disturbance in homeostasis may stem from exposure to environmental pollutants or hazardous laboratory chemicals. This study therefore examined the effects of carbon tetrachloride (CCl4), a common laboratory chemical on the levels of blood lipid molecules in animal model (Wistar rat). After a 14-day period of acclimatization, 36 adult male Wistar rats (mean weight of 185.2 ± 5.3g) were randomly assigned to three groups, (n = 12). Group i animals served as control while groups ii and iii animals were subjected to single intraperitoneal injection of CCl4 at doses of 1.0 and 1.5 mL/kg of body weight (BW) respectively. Four animals from each group were sacrificed at 24 and 48h post CCl4 administration; and blood samples were collected and used for estimation of lipid molecules, using clinical diagnostic kits (Randox®). Analysis of the data obtained showed that CCl4 at the investigated doses caused significant alterations in levels of blood lipids when compared to their baseline values in control animals. There was a positive correlation between CCl4-induced alteration and administered dosage as well as exposure time. In 24h post CCl4 administration (at a dose of 1.0 mL/kg BW), total cholesterol (T-chol), triacylglycerol (TAG) and low density lipoprotein (LDL) were significantly ($P < 0.05$) elevated by 20.92, 6.28 and 18.40% respectively. These values respectively increased to 63.16, 59.61 and 75.34% at 48h post CCl4 treatment. Increase in CCl4 dosage to 1.5 mL/kg BW caused further elevation in blood T-chol (85.57%), TAG (84.24%) and LDL (105.61%). The level of HDL was apparently not affected by CCl4 at the investigated doses within 24h post administration. However, at 48h post administration, CCl4 caused significant ($P < 0.05$) decline in high density lipoprotein (HDL) by 47.03 and 88.41% at 1.0 and 1.5 mL/kg BW respectively. The outcome of this study highlights the potential of carbon tetrachloride (CCl4) to cause morbid disturbances in lipid homeostasis and further affirms its toxicity as a common laboratory chemical. It is therefore recommended that individuals should take necessary precautions in handling of CCl4 and avoid exposure to the chemical.

Keywords: *Carbon Tetrachloride; Toxicity; Lipids; Wistar Rats*

Introduction

For various reasons, humans are continuously exposed to potentially toxic substances. Carbon tetrachloride (CCl_4) is a common laboratory toxicant. Various concentrations of the chemical have been used to induce different degrees of liver damage in experimental animals [1-3], thus, it is a well-known hepatotoxic agent. Its hepatotoxicity is underlined by free radicals generated during its metabolism. CCl_4 is readily activated by different variants of cytochrome P enzymes such as cytochrome (CYP) 2E1, CYP2B1 or CYP2B2 and possibly CYP3A, to form the trichloromethyl radical (CCl_3^-) [4,5]. This radical can bind to cellular molecules such as nucleic acids, proteins and lipids, impairing crucial cellular processes like lipid metabolism with the potential outcome of fatty degeneration (steatosis). Moreover, adduct

formation between (CCl_3^-) and DNA is thought to function as initiator of hepatic cancer [6]. CCl_3^- can also react with oxygen to form the trichloromethylperoxy radical (CCl_3OO^-) which initiates lipid peroxidation, a process which attacks and oxidized lipids in membranes. This consequently affects the permeability of membranes, resulting in loss of cellular structural and functional integrity with attendant cell damage [4]. Blood lipids either free or bound play significant role in overall body functions and homeostasis. It is clinically important that they exist within defined physiological concentration. Hence, disturbances in blood lipid homeostasis will have negative implications on human health. For instance, hyperlipidemia has been associated with cardiovascular diseases, hypertension and a number of metabolic disorders as a major risk factor. It is on this background, the present study sought to investigate the dose and time dependent effects of CCl_4 intake on blood lipids using Wistar rat as study model.

Materials and Methods

Collection and management of animals

Thirty-six adult male rats (mean body weight of 185 ± 5.2g) were purchased from the Animal Breeding Unit of the Department of Physiology, University of Ibadan. They were managed according to the according to the procedure outlined by the National Academy of Science published by the National Institute of Health [7], approved by the Animal Research Ethics Committee of Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan Nigeria, for the use of animals in research. The animals were handled humanely, kept in a wooden cage, placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were acclimatized for two weeks prior to commencement of study. The animals were subjected to natural photoperiod of 12 hours light and 12 hours dark cycle and provided rats pallets (Top feeds) and water *ad libitum.* All animal experiments were carried out without anesthesia during the study.

Experimental design and methods

The animals were randomly divided into three (3) groups containing 12 animals each. Group 1 animals served as control while groups 2 and 3 animals were subjected to single intraperitoneal injection of CCl_4 at doses of 1.0 and 1.5 mL/kg of body weight (BW) respectively. Four animals from each group were sacrificed by cervical dislocation at 24 and 48h post CCl_4 administration; and blood samples were collected and used for estimation of lipid molecules [total cholesterol (T-chol), Triacylglycerol (TAG), High density lipoprotein (HDL) and Low density lipoprotein (LDL)], using clinical diagnostic kits (Randox).

Statistical analysis

The statistical significance of difference between groups were analyzed with One-Way Analysis of Variance (ANOVA) followed by independent-sample t test, using a statistical software tool, Prism Graphpad (version 6.4) at 95% confidence level ($P < 0.05$).

Results

Table 1 shows the effects of CCl_4 intake on the levels of blood lipids in respect to dose and duration of exposure. Administration of CCl_4 caused significant ($P < 0.05$) elevation in the values of total cholesterol (T-chol), triacylglycerol (TAG) and low density lipoprotein (LDL) as well as concomitant decline in high density lipoprotein (HDL) when compared to the baseline values of these parameters in control animals. The effects of CCl_4 was noted to be dose and time dependent as increased dosage and exposure time of the chemical caused further deviations of blood lipid parameters from the control group values. In 24h post CCl_4 administration at a dose of 1.0 mL/kg BW, T-chol, TAG and LDL were significantly elevated by 20.92, 6.28 and 8.40% respectively. These values respectively increased to 63.16, 59.61 and

75.34% at 48h post CCl_4 treatment. Increase in CCl_4 dosage to 1.5 mL/kg BW caused further elevation in blood T-chol, TAG and LDL by 85.57, 84.24 and 105.61% respectively. The level of HDL was apparently not affected by CCl_4 at the investigated doses within 24h. However, at 48h post administration, CCl_4 caused significant ($P < 0.05$) decline in HDL by 47.03 and 88.41% at 1.0 and 1.5 mL/kg BW respectively.

	Levels of blood lipids (mg/dL) 24 h Post CCl_4 Administration				Levels of blood lipids (mg/dL) 48 h Post CCl_4 Administration			
Group	**T-CHOL**	**TAG**	**HDL**	**LDL**	**T-CHOL**	**TAG**	**HDL**	**LDL**
Control	96.17 ± 8.47	112.75 ± 9.87	43.21 ± 5.32	35.32 ± 4.12	96.17 ± 8.47	112.42 ± 9.87	43.21 ± 5.32	35.32 ± 4.12
Dose 1 1.0 mL/kgBW	[b]116.29 ± 6.54	[b]119.83 ± 3.46	42.19 ± 3.71	41.82 ± 2.78	[d]156.91 ± 9.35	[d]179.43± 7.23	[d]22.89 ± 5.12	[d]61.93 ± 4.32
Dose 2 1.5 mL/kgBW	118.29 ± 9.11	123 ± 5.25	44.21 ± 6.67	[c] 81.22 ± 8.17	[e]178.46 ± 11.54	[e] 207.12 ± 9.19	[e]5.01 ± 2.37	[e] 72.62 ± 5.49

Table 1: *Effects of CCl_4 administration on blood lipid levels in respect to dose and time. Values are expressed as mean ± standard deviation (SD) of 4 rats. T-chol: Total Cholesterol; TAG: Total Triacyl Glycerol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein. Statistical significance was analyzed at 95% confidence level ($P < 0.05$) with One-Way Analysis of Variance (ANOVA) followed by independent t test using a statistical software, Graphpad Prism, version 6.4, b = Significant when compared to control, c = Significant when compared to dose 1, d = Significant when compared to control and post 24h corresponding value, e = Significant when compared to dose 1 and post 24h corresponding value.*

Discussion

The significance of lipid molecules in the functional integrity of living organisms cannot be over stressed. These molecules besides been efficient source of energy perform several other vital functions ranging from metabolic to cell signaling in the body [8]. It is clinically important that they exist within defined physiological concentrations. Hence, a disturbance or compromise in their homeostasis or metabolism can result in a number of metabolic disorders or disease conditions which could be life-threatening [9]. Unfortunately, and for various reasons, humans are continuously exposed to potentially toxic substances. Carbon tetrachloride (CCl_4) is a common laboratory toxicant. Its hepatotoxic effects and damaging impact on other organs have been established by previous studies [10,11]. The present study investigated its effects on blood lipids at different doses and different period of exposure.

The results obtained showed clearly that CCl_4 intake by experimental animal's impact negatively on their lipid homeostasis. CCl_4 markedly altered the levels of total cholesterol (T-chol), triacylglycerol (TAG), high density lipoproteins (HDL) and low density lipoproteins (LDL). Elevated concentration of T-chol, TAG and LDL as observed in this study following CCl_4 intake has been implicated in the incidence of a number of disorders and diseases, particularly cardiovascular diseases and hypertension. The ability of CCl_4 to cause significant alterations in the levels of blood lipids is likely associated with its pro-reactive species production during metabolism. CCl_4 is known to generate free radicals including trichloromethyl radical (CCl_3^-) and trichloromethyl peroxyl radical (CCl_3OO^-) when metabolized in the body [4,5,12]. The generated radicals are arguably responsible for the disturbance in lipid metabolism or homeostasis as noted in this study. Moreover, it has been reported that free radicals have the ability to attack lipid molecules by binding directly to them or indirectly to other molecules important for lipid metabolism, with the potential outcome of fatty degeneration (steatosis) [8,9]. Formation of CCl_3OO^- which occurs from the reaction of CCl_3^- with oxygen apparently account for huge part of CCl_4 damaging effects on lipids. Trichloromethylperoxy radical (CCl_3OO^-), a highly reactive species initiates the chain reaction of lipid peroxidation, a process which attacks and destroys

polyunsaturated fatty acids component of membranes, especially those associated with phospholipids. Consequently, the permeability of mitochondrial, endoplasmic reticulum, and plasma membranes is compromised, leading to alteration in cellular calcium sequestration and homeostasis, which in turn contribute heavily to cell damage [15,16]. The present study though examined the effects of CCl_4 on blood lipids in animals (Wistar rats); it is however very important to note that the findings herein are highly applicable to humans.

Conclusion

This study shows the potential of carbon tetrachloride (CCl_4) to cause serious compromise in blood lipid homeostasis and supports previous reports on its toxicity as a common laboratory chemical. We therefore recommended that necessary precautions should be observed in handling of CCl_4 and exposure to the chemical should be avoided.

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