

EFFECT OF DIFFERENT VEHICLES ON HEPATO- PROTECTIVE EFFICIENCY OF DESFERRIOXAMINE IN RADIATED CARBON TETRACHLORIDE TREATED MICE

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Abstract

The effect of desferrioxamine (DFO) in different vehicle (Aqueous and oily) against hepatotoxicity induced by carbon tetrachloride (CCl₄) in irradiated mice and irradiated carbon tetrachloride (IR-CCl₄) in normal mice was investigated. A single dose of CCl₄ and IR-CCl₄ (20 µl/kg, i.p.) in irradiated mice (IR-mice) and normal mice induced hepatotoxicity, manifested biochemically by significant elevation of serum enzyme activities, such as alanine transaminase (ALT, EC:2.6.1.2) and aspartate transaminase (AST, EC:2.6.1.1). Hepatotoxicity was further evidenced by significant decrease of total sulfhydryl (-SH) content, and catalase (EC: 1.11.1.6) activity in hepatic tissues and significant increase in hepatic lipid peroxidation measured as malondialdehyde (MDA). Pretreatment of normal mice and IR-mice with DFO (200 mg/kg i.p dissolved either in water or arachis oil vehicle) 1 h before CCl₄ or IR-CCl₄ injection ameliorated the hepatotoxicity, as evidenced by a significant reduction in the elevated levels of serum enzymes as well as a significant decrease in the hepatic MDA content and a significant increase in the total sulfhydryl content 24 h after CCl₄ or IR-CCl₄ administration.. These results indicated that both of oily

and watery DFO can effectively ameliorated the hepatotoxicity induced by CCl_4 in IR-mice or IR- CCl_4 in normal mice. Although, the efficiency of the hepatoprotective effect of DFO in oily vehicle was higher than that DFO in aqueous vehicle. The hepatoprotective effect of DFO possibly through inhibition of the production of oxygen free radicals that cause lipid peroxidation.

Key Words:

Desferrioxamine, Oil vehicle, Water vehicle, Irradiated mice, Irradiated carbon tetrachloride, Hepatotoxicity, Lipid peroxidation.

Introduction

Irradiation is a complex phenomenon in which molecules are excited, ion pairs are formed, and chain reactions are completed by free radicals [1]. Radiation offers several advantages as a sterilization method that makes it attractive in growing number of situations. Articles sterilized by radiation include a large variety of medical products [2,3]. The extent and nature of the damage produced in organic substances by gamma radiation was attributed to radicals produced by the irradiation [4].

Carbon tetrachloride (CCl_4) is used as solvent for oils, fats, lacquers, varnishes, rubber waxes, resins; extracting oil from flowers, seeds [5], ibuprofen in human serum [6], free chlorpheniramine in pharmaceutical preparation [7], cyclopropane for determination of cyclopropane solubility coefficients [8] and as starting material in manufacturing of many organic compounds [5].

CCl_4 is a xenobiotic which produces hepatotoxicity in humans as well as in animals [9,10]. The hepatotoxic effect of CCl_4 is thought to result from its reductive dehalogenation

by cytochrome P-450 enzyme system to the highly reactive free radical, trichloromethyl radical $\cdot\text{CCl}_3$ [11]. This radical quickly adds molecular oxygen to form trichloromethylperoxy radical [12]. Removal of hydrogen atoms from unsaturated fatty acids by such radical created carbon-centered lipid radicals [11]. These lipid radicals quickly add molecular oxygen to form lipid peroxy radicals, thereby, initiating the process of lipid peroxidation. Unless scavenged by radical scavengers, these lipid peroxy radicals in turn abstract hydrogen atoms from other lipids molecules, thereby propagating the process of lipid peroxidation [13]. Iron can catalyze oxygen free radical reactions that lead to peroxidation of membrane lipids or inactivation of antioxidant enzyme activities [14], and consequently damage of microsomes, mitochondria and nuclei that lead to impaired physiological functions of hepatocytes [15]. Also, it has been reported that iron plays a role as a mediator of CCl_4 -hepatotoxicity [16].

Administration of some antioxidants such as cysteamine [17], silymarin [12], tetrahydroneopeterin [18], schisandrin B [19] and vitamin E [20] has been shown to significantly suppress CCl_4 -induced hepatotoxicity in mice. Desferrioxamine is an iron chelator that is mainly used to treat iron poisoning [21]. The iron chelating properties of DFO has been shown to play a primary role in protecting the liver against CCl_4 -induced hepatotoxicity [22]. It has been demonstrated that administration of DFO prevents lipid peroxidation induced by CCl_4 [16]. Also, it has been reported that DFO could significantly decrease LDH leakage and reactive oxygen species production by cyanobacteria extract-induced hepatotoxicity in primary cultured rat hepatocyte [23]. Previously, we have shown that DFO attenuated hepatotoxicity induced by CCl_4 [24].

Therefore, the goal of the present study was to investigate whether DFO can attenuate hepatotoxicity induced by IR- CCl_4 and CCl_4 in normal and IR-mice respectively.

In addition, whether using different vehicles (water or arachis oil) can affect the hepatoprotective efficiency of DFO.

Experimental

Chemicals:

Thiobarbituric acid (TBA) and arachis oil were a product of Fluka (Buchs, Switzerland). DFO (Desferal) and carbon tetrachloride were obtained from Ciba Geigy (Basel, Switzerland) and Aldrich Chemical Company (Milwaukee, WI, USA) respectively. All the remaining chemicals were of the highest analytical grade.

Animals:

Male Swiss albino mice weighing 22-25 g were used in all experiments. They were obtained from the Experimental Animal Care Center of King Saud University, Riyadh, KSA. Animals were maintained under standard conditions of humidity with regular light/dark cycle and free access to food (Purina Chow) and water.

Gamma radiation:

A. Radiation of carbon tetrachloride

50 ml of carbon tetrachloride (CCl₄) were filled into plastic tubes and were exposed to gamma radiation from Cobalt-60 source in a Gammacell 220 (Nordion International Inc., Kanata, Canada). The selected dose of the radiation in the present study was 25 kGy depending on our own preliminary experiment. The dose rate was 1.15 Gray/second. The exposure time was 6.04 hrs. The zero dose (non-radiated CCl₄) provided as a control on temperature/ storage/ transportation effects on the samples.

B. Radiation of mice

A group of mice are subjected to the same conditions of radiation as in CCl₄ procedure. The selected dose of radiation in the present study was 50 Gray. The dose rate was 1.15 Gray/ second and the exposure time was 1 min.

Experimental protocol:

CCl₄ (0.2 % v/v) was dissolved in corn oil and a dose of 20 µl/kg of CCl₄ was intraperitoneally administered to mice to induce liver damage. The rise in serum ALT was taken as evidence for impaired liver function. In a preliminary experiment, CCl₄ was administered to IR-mice and IR-CCl₄ in normal mice at different doses ranging from 10 to 25 µl/kg and the dose of 20 µl/kg was selected in the present model, since it produces moderate hepatotoxicity. Thus the protective effects of DFO could be evaluated. In our own previous study, the peak of an increase in serum ALT activity, an index of liver injury, in mice receiving CCl₄ alone, was observed at 24 h and started to decrease after 48 and 72 h [24]. A portion of DFO was dissolved in aqueous vehicle while the other was suspended in oily vehicle.

The animals were divided into 9 groups, the first group (control) received corn oil (10 ml/kg i.p), the second group IR-mice with 50 Gray. The third group received CCl₄, (20 µl/kg i.p). The fourth group was given IR- CCl₄, 25 kGy (20 µl/kg i.p). The fifth group was IR-mice with 50 Gray treated with 20 µl/kg CCl₄. The last four groups were given DFO in oily and aqueous vehicle 1 h before CCl₄ and IR-CCl₄ injection in IR-mice or normal mice. In the present study, the dose of 200 mg/kg of DFO was selected, since it showed the optimum hepatoprotective effect against CCl₄ as evidenced from a significant reduction of the serum enzymes tested.

At 24 h after of CCl₄ dosing, blood samples were drawn from the orbital plexus, under light ether anaesthesia, into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min at 4000 rpm and stored at -20°C until analysis. The liver was isolated, washed with saline, weighed and homogenized in ice cold saline, and then 10% homogenate of the liver was made in a Branson sonifier (250, VWR Scientific, Danbury, Conn., USA). Serum ALT and AST were determined kinetically as described earlier by Reitman and Frankul [25] using commercially available diagnostic kits (Biosystems, Barcelona, Spain). Malondialdehyde (MDA) production as an index of lipid peroxidation, total sulfhydryl content and catalase activity in the liver tissues were determined according to the methods of Ohkawa et al [26], Ellman [27] and Higgings et al [28], respectively.

Statistical analysis: Data are expressed as (means ± SEM). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Significance was accepted at P < 0.05.

Results

Effect of DFO on hepatotoxicity induced by CCl₄ and IR-CCl₄ in IR-mice and normal mice respectively.

Table 1 and 2 shows that i.p. injection of CCl₄ and IR-CCl₄ (25 kGy) in IR-mice and normal mice induced hepatotoxicity. Serum ALT and AST were significantly increased. Pretreatment with DFO (200 mg/kg i.p) dissolved in arachis oil vehicle prevent the significant increase in serum enzymes activities. Serum ALT and AST were significantly decreased nearly to the normal value in both groups treated (normal mice treated with IR-CCl₄ or IR-mice treated with CCl₄). However, administration of DFO in aqueous vehicle

ameliorated hepatotoxicity in both groups (IR-CCl₄ in normal mice and CCl₄ in IR-mice). These were evidenced by the significant reduction in serum enzymes activities, ALT and AST. However,, it was still significant from control.

Effects of DFO (aqueous and oily) on lipid peroxide (MDA), reduced glutathione (GSH) and catalase activity in hepatic tissues.

Table 3 and 4 demonstrates that administration of IR-CCl₄ or CCl₄ in normal or IR-mice induced hepatotoxicity as evidenced by significant elevation of lipid peroxide measured as MDA and significant reduction of reduced glutathione. Pretreatment with DFO (oily) significantly decreased its elevated level of lipid peroxide in both groups (CCl₄ in IR-mice and IR-CCl₄ in normal mice). In addition, it prevents the significant reduction of reduced glutathione induced by CCl₄. Administration of

Tab. 1. Effects of DFO on serum ALT and AST enzymes activities of mice injected with IR-CCl₄. DFO (200 mg/kg i.p.) was given either in aqueous and oily vehicle 1 h prior to IR-CCl₄(20 µl/kg) injection.

	Serum (M ± SE)	
	ALT (U/ml)	AST (U/ml)
Control	81 ± 9.2	147 ± 17
CCl ₄ + normal mice	2335±467*	1750 384*
IR-CCl ₄ + normal mice	2392± 682*	1900 ± 351*
DFO (oily) + IR-CCl ₄ +normal mice	170 ± 12#	282 ± 89*#
DFO (Aq.) + IR-CCl ₄ +normal mice	1327 ± 279*	624 ± 185*#

* Significantly different from control group

Significantly different from corresponding group

#* P<0.05

aqueous DFO attenuated the rise in lipid peroxide induced by CCl₄ in both groups (CCl₄ in IR-mice and IR-CCl₄ in normal mice) and prevent the significant decrease in reduced glutathione. However, there was no significant change in catalase activity in hepatic tissues.

Tab.2. Effects of DFO on serum ALT and AST enzymes activities of IR-mice injected with CCl₄. DFO (200 mg/kg i.p.) was given either in aqueous and oily vehicle 1 h prior to CCl₄ or IR-CCl₄(20 µl/kg) injection.

	Serum (M ± SE)	
	ALT (U/ml)	AST (U/ml)
Control	81 ± 9.2	147 ± 17
IR-mice	99.4 ± 8.2	207 ± 8.5
CCl ₄ and (IR-mice)	3800 ± 1311*	3062 ± 485*
DFO (oily) + CCl ₄ +IR-mic	220± 12#	180 ± 4#
DFO (Aq.) +CCl ₄ + IR-mice	994 ±210#	631± 17*#

* Significantly different from control group

Significantly different from corresponding group

#* P<0.05

Discussion

The results of the present study indicate that the DFO can protect the hepatotoxicity induced by a single administration of IR-CCl₄ (20 µl/kg, i.p.) in male Swiss albino mice and

CCl₄ (20 µl/kg, i.p.) in IR-mice. Our previous study demonstrated that treatment of mice with DFO (200 mg/kg, i.p.) neither induced any change in the activities of serum ALT and AST measured nor influenced liver lipid peroxide, total sulfhydryl contents and catalase activity [24]. In the present study, pretreatment with

Tab.3. Effects of DFO on hepatic lipid peroxide and total sulfhydryl contents and catalase activity in liver homogenates of mice injected with IR-CCl₄. DFO (200 mg/kg i.p.) was given either in aqueous and oily vehicle 1 h prior to IR-CCl₄(20 µl/kg) injection.

	Tissues homogenates (M ± SE)		
	MDA (nmol/g liver)	GSH (µmol/g liver)	Catalase (mmol/min/g liver)
Control	360 ± 30	6.5 ± 0.3	123 ± 8.5
CCl ₄	1132 ± 184*	2.7 ± 0.3*	114 ± 21.6
IR-CCl ₄	1261 ± 149*	3.1 ± 0.5*	100 ± 8.5
DFO (oily) + IR-CCl ₄ +normal mice	706 ± 127*	5.2 ± 0.3	155 ± 12.5
DFO (Aq.) + IR-CCl ₄ +normal mice	455 ± 59#	7.6 ± 0.4#	109 ± 14.3

* Significantly different from control group

Significantly different from Corresponding group

#* P<0.05

DFO (200 mg/kg, i.p.) either dissolved in aqueous or oily vehicle 1 h before CCl₄ injection in IR-mice or IR-CCl₄ (20 µl/kg, i.p.) in normal mice ameliorated hepatotoxicity. This was evidenced by a significant reduction in activities of serum enzymes, ALT and AST. Also a significant reduction in lipid peroxidation and a significant increase in total sulfhydryl

contents were observed in the liver of CCl₄-injected mice pretreated with DFO. Although an aqueous vehicle is generally

Tab.4. Effects of DFO on hepatic lipid peroxide and total sulfhydryl contents and catalase activity in liver homogenates of IR-mice injected with CCl₄. DFO (200 mg/kg i.p.) was given either in aqueous and oily vehicle 1 h prior to CCl₄(20 µl/kg) injection.

	Tissues homogenates (M ± SE)		
	MDA (nmol/g liver)	GSH (µmol/g liver)	Catalase (mmol/min/g liver)
Control	360 ± 30	6.5 ± 0.3	123 ± 8.5
IR-mice	320 ± 80	7.74 ± 0.5	94.8 ± 8.7
CCl ₄ and (IR-mice)	1388 ± 311*	4.4 ± 0.6*	96 ± 13.2
DFO (oily) + CCl ₄ + IR-mice	465 ± 96#	6.8 ± 0.6#	122 ± 11.2
DFO (Aq.) + CCl ₄ + IR-mice	472 ± 46#	9.5 ± 1#	119 ± 11.2

* Significantly different from control group

Significantly different from Corresponding group

#* P<0.05

preferred for an injection, however, its use may be precluded in a formulation due to limited water solubility of a medicinal substances or a prolonged action is required [29]. Therefore, a non-aqueous solvent presently employed in parenteral products such as fixed oil, glycerine, polyethylene glycol. In the present study, i.p administration of oily DFO resulted in highly amelioration of the hepatotoxicity induced by IR-CCl₄ in normal mice and

CCl₄ injection in IR-mice, as the level of the serum enzymes were reduced back almost to the normal. In addition, it prevents the rise in hepatic lipid peroxide.

The biochemical mechanism involved in the development of CCl₄ hepatotoxicity has been investigated. It is generally believed that this toxicity is due to lipid peroxidation caused by carbon trichloromethyl radical, CCl₃ [30]. In the present study, the development of liver cell injury by a single administration of CCl₄ in IR-mice or IR-CCl₄ in normal mice (20 µl/kg, i.p.) was associated with a significant increase in hepatic lipid peroxide content and a significant decrease in hepatic total sulfhydryl content, a potent factor in the control of lipid peroxidation [31]. These findings are consistent with a previous report [32].

The hepatoprotective effect of DFO against CCl₄ (20 µl/kg, i.p.) or IR-CCl₄-induced hepatotoxicity, as demonstrated by the significant reduction of increased serum ALT and AST was associated with a parallel significant inhibition of elevated hepatic lipid peroxide content and a significant increase in total sulfhydryl content in liver tissues. These findings may indicate that inhibition of lipid peroxidation is the initial event in the mechanism by which DFO ameliorates CCl₄ or IR-CCl₄-induced hepatotoxicity and is compatible with the study demonstrating that CCl₄ toxicity could be ameliorated by pretreatment with inhibitors of CCl₄ metabolism [33] or antioxidants [34]. This does not, however, support the work of Stacy and Klassen [35] who have reported that inhibition of lipid peroxidation can not prevent CCl₄-induced cellular injury in isolated rat hepatocytes. However, in our previous study, DFO was a potent lipid peroxidation inhibitor in an *in vitro* study using liver homogenates of ¹normal mice as a source of lipids and Fe³⁺/ascorbate to induce lipid peroxidation [24].

In the present study DFO protected the liver of the mice against CCl₄-induced hepatic damage. This finding is in harmony with the previously reported finding showed that DFO inhibits adriamycin-induced hepatotoxicity by suppression of lipid peroxidation [36]. Moreover, it has been shown that DFO could significantly decrease LDH leakage and reactive oxygen species production by cyanobacteria extract-induced hepatotoxicity in primary cultured rat hepatocytes [23]. In addition, the antidotal effects of DFO in experimental liver injury were studied by Siegers et al [22]. The authors have demonstrated that DFO can protect against hepatotoxicity induced by CCl₄, but not by bromobenzene, although a marked in vivo lipid peroxidation is observed, that appears to be iron-dependent, and have indicated that lipid peroxide is not mainly responsible for bromobenzene-induced hepatotoxicity.

In conclusion: The results of the present study clearly demonstrate that both of oily and watery DFO can effectively ameliorated the hepatotoxicity induced by CCl₄ in IR-mice or IR-CCl₄ in normal mice. Although, the efficiency of the hepatoprotective effect of DFO in oily vehicle was higher than that DFO in aqueous vehicle. Further studies are needed to explore the possible mechanisms of cytoprotective actions.

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