Effect of benfotiamine on hepatic tissue levels of free calcium, copper, iron and zinc during CCl4-induced hepatotoxicity in rats

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Abstract

Background and Objectives: Toxic injury occurs in the liver more often than other organ, this can be attributed to the fact that virtually all ingested substances that are absorbed are first presented to the liver and that the liver is responsible for the metabolism and elimination of many substances. Carbon tetrachloride (CCl4) is very well known to cause hepatotoxicity that may be associated with impaired calcium and trace element homeostasis. This study was designed to evaluate the protective effect of benfotiamine against CCl4-induced disturbances in calcium, iron, copper and zinc homeostasis in liver tissue of rats.

Methods: Liver tissue homogenate from normal controls, CCl4-treated and benfotiamine (70 mg/kg) pre-treated before induction of hepatic damage with CCl4 in rats were obtained, and processed for estimation of levels of free forms of calcium, iron, copper and zinc using atomic absorption spectrophotometry.

Results: Analysis of data revealed significant elevation in calcium, iron and copper levels in hepatic tissue due to exposure to CCl4 compared to controls, while zinc levels not significantly affected. Pretreatment with benfotiamine results in significant decrease in calcium, iron and copper levels compared to non-treated group, while zinc levels found to be significantly elevated.

Conclusions: Benfotiamine has a protective effect against CCl4-induced hepatic tissue damage which may be, in part, attributed to restoration of calcium and other trace elements homeostasis.

Key words: benfotiamine, CCl4, hepatotoxicity, calcium, trace elements

Introduction

Many chemicals, including carbon tetrachloride (CCl4), have long been known to induce hepatotoxicity [1]. High doses and/or long-period exposure are necessary in animals to induce various types of hepatic tissue damage, like parenchymal cell degeneration or cholestatic damage [2]. At this stage, a large number of structural, metabolic and functional alterations are observed in hepatic tissue [3]. The mechanism of degeneration or necrosis remains unsettled, and cannot be traced to single, well determined causes [4]. Among the suggested mechanisms are the impaired calcium homeostasis [5], and the involvement of highly reactive radical species that play a role in the hepatotoxicity of CCl4, and considered as a possible mechanism that may contribute to cellular damage [6]. Many oxidant species can interact with both cellular metabolites and structural elements modifying their properties by changing calcium ion homeostasis, with consequent modification of essential life processes [7]. Essential trace elements like iron, copper and zinc are necessary cofactors for the activities of many antioxidant enzymes like superoxide dismutase [8], catalase and heme oxidase [9], which are critical for cytoprotection against reactive radicals-induced hepatotoxicity; disturbances in the

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cellular or tissue status of these trace elements may predispose to toxicity [10]. *Benfotiamine* (S-benzoyl thiamine-O- monophosphate) is a synthetic derivative of thiamine (vitamin B1); it has many effects including reduction of glucose toxicity [11], alleviation of diabetes-induced cerebral oxidative damage [12], acceleration of the healing of ischemic diabetic limbs in mice[13] and rescue of cardiomyocyte contractile dysfunction in experimental diabetes mellitus [14]. The present study was designed to evaluate the effect of benfotiamine on hepatic tissue levels of free forms of calcium, iron, copper and zinc during CCl4-induced hepatotoxicity in rats.

Methods

Twenty-four male Wistar rats (60 days old, weighing 200-250 g) purchased from the animal house in the college of Pharmacy/ University of Baghdad were used in the experiments. The animals were handled in the animal facilities of the College of Pharmacy in Sulaimani under standard laboratory conditions of a 12-hour light/dark cycle and fixed temperature (25±2˚C). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with the U.S. National Institutes of Health's Guide for the Care and Use of Laboratory Animals with the approval of the local ethics committee of University of Sulaimani. The animals were randomly allocated to one of the three experimental groups (*n* =8): group 1 served as the control and received saline, and groups 2 and 3 were treated with either normal saline (2 ml/kg) or Benfotiamine (70 mg/kg/day) respectively for 7 days before induction hepatotoxicity with single oral dose of CCl4 (2 ml/kg). On day 8, the animals were sacrificed and one gram of liver was taken to prepare 10% tissue homogenate, and utilized for assessment of tissue levels of free calcium, iron and the trace elements copper and zinc using atomic absorption spectrophotometer (Pye-Unicam Ltd, England); each sample was diluted with de-ionized water to bring the concentrations of the studied elements within the working range of the atomic absorption apparatus [15]. The values were compared with standard solutions of the studied elements specially prepared for this purpose. Analysis of variance followed by Tukey's test was used to test the significance of differences between treatments; *P*-value of 0.05 was considered significant.

Results

The results presented in table 1 showed that oral administration of 2ml/kg CCl4 produced significant elevation ($P<0.05$) in hepatic tissue levels of calcium (108%), iron (38%) and copper (109%) compared to control group, while hepatic tissue zinc levels did not significantly affected ($P>0.05$). Pretreatment of animals with 70mg/kg benfotiamine orally, before induction of hepatotoxicity with CCl4, resulted in significant reduction ($P<0.05$) in hepatic calcium levels (38%) compared to CCl4 only treated group; this level was found to be significantly higher than that reported in controls. Meanwhile, benfotiamine reduces both free iron and free copper levels in hepatic tissues (22% and 57% respectively) compared to non-treated group and comparable to those reported in control group; while zinc levels were significantly elevated (21%) to values higher than those reported in control group.

Table 1. Effect of pre-treatment with benfotiamine on hepatic tissue levels of the free forms of calcium, iron, copper and zinc during CCl4-induced hepatotoxicity in rats

Treatment groups	Hepatic tissue levels µg/g tissue			
	Free Calcium	Free Iron	Free Copper	Free Zinc
Control n=8	3.7 ± 0.21[a]	4.1 ± 0.55[a]	1.1 ± 0.12[a]	1.5 ± 0.13[a]
CCl4 + saline n=8	7.7 ± 0.42[b]	5.4 ± 0.36[b]	2.3 ± 0.11[b]	1.4 ± 0.09[a]
CCl4+Benfotiamine 70mg/kg n=8	4.8 ± 0.39[c]	4.2 ± 0.40[a]	1.0 ± 0.08[a]	1.7 ± 0.11[b]

Values presented as mean ± SEM; n= number of animals; values with non-identical superscripts (a, b, c) within the same parameter were considered significantly different (*P*<0.05).

Discussion

The toxicity of many xenobiotics, including CCl4, is associated with the production of free radicals, which are toxic and implicated in the pathophysiology of many diseases [16]. In the present study, administration of CCl4 elevates free calcium levels in hepatic tissue compared to control group. It is well known that calcium acts as a second messenger and has an important role in a number of cell functions, such as differentiation, proliferation, contraction, migration, apoptosis, and protein synthesis [17]. Exposure of hepatocytes to toxic insult with CCl4 results in drastic increase in intracellular calcium due to excessive entry or release from intracellular compartments with consequent initiation of primary cellular damage [18]. Moreover, other mechanisms including kupffer cells activation, plasma membrane damage and release of inflammatory cytokines may be involved in the maintenance and aggravation of hepatocytes damage [19, 20]. Copper and iron are important trace elements for normal cell function, they are the components of several proteins and enzymes involved in a variety of metabolic pathway [21,22]; meanwhile, when liberated in free form, due to cytotoxicity and oxidative stress, they can be considered potentially toxic and interfere with many vital physiological processes with consequent degeneration and cellular death [23]. In the present study, exposure to CCl4 elevates tissue levels of free copper and iron due to release from stores and sequestering mechanisms, an event that precede cytotoxicity and tissue damage. When copper and iron are not functionally or tightly bound to sequestering proteins, they can, as part of a low molecular mass complex, catalyze unwanted electron transfer reactions with consequent formation of reactive and damaging species such as hydroxyl radical [24]; they can induce oxidative stress by catalyzing the conversion of superoxide and hydrogen peroxide to more potent oxidants such as hydroxyl radical, which can cause tissue injury by initiating lipid peroxidation and oxidation of proteins and nucleic acids [25]. In the present study, the reported increase in the levels of copper and iron due to exposure to CCl4 is consistent with other studies [26]. Zinc is an important trace element for the activity of many enzymes and involved in many cellular processes including cell proliferation, differentiation, and apoptosis; it also takes part in the function of the immune system, intermediary metabolism, DNA metabolism and repair [27]. In the present study, although hepatic tissue levels of zinc are not significantly changed as a result of exposure to CCl4, impairment of its proper binding with functional proteins and/or improper homeostasis may be the cause behind improper repair and consequent cellular death. The results presented in (table 1) indicated that pre-treatment with

benfotiamine of rats challenged with toxic dose of CCl4 restores tissue levels of calcium, iron and copper, in association with significant elevation in zinc levels. Benfotiamine showed an intrinsic antioxidative activity by itself [28]; moreover, the use of benfotiamine results in increased intracellular thiamine diphosphate levels, a cofactor of transketolase enzyme, where its activation by thiamine may reduce production of superoxide and other reactive species through activation of the pentose phosphate pathway [29,30]. It has been reported also that pre-treatment with benfotiamine increased GSH levels in hepatic tissue and offer a good protection against lipid peroxidation that induced by CCl4 [31]. Additionally, previous studies have shown that benfotiamine is capable to inhibit the advanced glycation end-product formation pathway and to completely prevent diabetes-induced glycoxidation products in peripheral nerves of diabetic rats [32, 33, 29]; it also reduces the oxidative stress and consequently improves the integrity of vascular endothelium and enhances the generation of nitric oxide to prevent nicotine and uric acid induced experimental vascular endothelial dysfunction [34]. Accordingly, by activating transketolase enzyme, benfotiamine can block the biochemical pathways that predispose to oxidative stress [29, 35], and one of the markers for these effects may be the restoration of calcium and other trace element homeostasis. In conclusion, benfotiamine has a protective effect against CCl4-induced hepatic tissue damage which may be, in part, attributed to restoration of calcium and other trace elements homeostasis.

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