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The Effects of L-Arginine in Modulating Liver Antioxidant Biomarkers within Carbon Tetrachloride Induced Hepatotoxicity: Experimental Study in Rats

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ABSTRACT

Arginine is an amino acid, aid in liver detoxication. Carbon tetrachloride (CCl_4) induces hepatic injury by the initiation of lipid peroxidation process through the formation of free radicals, leading to liver damage. The present work aimed to investigate whether the administration of arginine had protective and/or curative effects against hepatic lipid peroxidation induced by CCl_4 in rats. Sixty Swiss albino rats included in the present study were subdivided into four groups equally: (1) control group, (2) CCl_4-intoxicated group; rats were injected intraperitoneally (i.p.) with CCl_4, (3) protection group; rats were pre-treated with arginine for 6 days, then injected i.p. with CCl_4 and (4) curative group; rats were injected i.p. with CCl_4, 24 hours later, rats were post-treated with arginine for six days. Rats were sacrificed at the end of the treatment and liver tissues were obtained, homogenized and used for biochemical analyses. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase, glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S- transferase (GST) and reduced glutathione (GSH) levels were assayed by spectrophotometry. CCl_4 intoxication increased the level of hepatic MDA, decreased the level of hepatic GSH and inhibited the activity of antioxidant enzymes (GPx, GR, GST, SOD and catalase) versus their levels in the normal group. Pre- and post-treatment with arginine decreased hepatic level of MDA, increased GSH level and improved the activities of all antioxidant enzymes compared to the untreated CCl_4–intoxicated rats. Arginine administration have hepetoprotective and hepatocurative effects against CCl_4 induced lipid peroxidation, oxidative stress and liver damage, also, the curative effects of arginine were found to be more effective than its protective effects.

Keywords: Arginine, Lipid peroxidation, Antioxidant enzymes, Liver damage, Carbon tetrachloride (CCl_4)

INTRODUCTION

L-arginine is a diamino monocarboxylic amino acid. It is classified as a nonessential amino acid; however in certain situation such as sepsis, stress and trauma, it can become semi-essential or conditionally essential[1]. Arginine is a precursor for synthesis of proteins, nitric oxide, creatine and polyamines, and it is an intermediate in ammonia detoxification via ornithine cycle. Arginine has been linked to enhanced immunity, the release of human growth hormone, greater muscle mass, rapid healing from injury, increased sexual potency, and helping to reverse atherosclerosis[2-4]. In adult humans, the endogenous synthetic capacity for arginine, amounting to approximately to 20% of the daily requirement, which is relatively small compared to the daily need, hence, a dietary supplement of arginine becomes indispensable under conditions of increased demand such as growth[5], and tissue repair[6] or decreased dietary supply[7]. Arginine has been reported to have

immuno-supportive effects, especially under catabolic conditions[8]. Also, it helps in liver detoxification by neutralizing ammonia, and may benefit in the treatment of liver disorders such as liver cirrhosis and fatty liver[9].

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins, it causes liver damage following its cleavage by cytochrome P450 forming trichloromethyl free radical (CCl_3), which quickly adds molecular oxygen to form the trichloromethyl peroxyl radical[10]. The removal of hydrogen atoms from unsaturated fatty acids of lipids by such free radicals creates carbon-centered lipid radicals which add molecular oxygen to form lipid peroxyl radicals initiating lipid peroxidation. Unless, scavenged by vitamin E or other radical scavengers, the radicals propagate the process of lipid peroxidation[11]. CCl_4 administration induced acute liver damage in rats (16-24 hours after administration). An increase in the levels of plasma amino acids was observed for most of individual amino acids except arginine which decreased in a dose dependent manner[12].

Cellular protection mechanisms against free radicals involve several antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and low molecular weight substances such as glutathione. L-arginine can prevent alloxan-induced â-cell damage, and the development of diabetes, and restore the antioxidant status to near normal levels[13]. Pre-treatment of rats with L-arginine before the administration of cyclosporine (nephrotoxic drug) prevented the significant increase in malondialdehyde (MDA), and greatly improve the activity of GPx enzyme. Additionally, L-arginine ameliorated the depletion of glutathione content. These findings may indicate a possible protective effect of L-arginine against nephrotoxicity induced by cyclosporine treatment[14]. Furthermore, L-arginine co-supplementation prevented the retention of calcium oxalate crystals by way of protecting the renal cells from oxidative damage[15].

The aim of this study is to investigate the effects of L-arginine administration on modulating the antioxidant biomarkers, the possible protective and curative roles in induced liver damage by carbon tetrachloride.

MATERIALS AND METHODS

Animals

A total of 60 Inbred mature albino rat (Sprague Dawley strain), average weight 130-150g, were obtained from the animal facility of The Faculty of Medicine, Mutah University. They were acclimatized under standard conditions 12:12 light: dark cycle at room temperature 24±1°C, and 50%±10% relative humidity for four weeks prior to the experiments and had access to food and water *ad libitum*. The experiments were conducted according to the ethical norms approved by the Faculty Ethics Committee. The rats were randomly distributed in four groups (fifteen rats in each group):

Group I (control group): received normal diet (rat chow)

Group II (CCl_4-intoxicated group): rats were injected i.p. with a single dose of 2% CCl_4 in vegetable oil (1 ml/kg body weight) and were sacrificed by decapitation after 24 hours of CCl_4 injection.

Group III (protection group): rats were received oral administration of 200 mg L-arginine in (1 ml tween/kg body weight) daily for six days, followed by i.p injection with a single dose of 2% CCl_4 in vegetable oil (1 ml/kg body weight) and were sacrificed by decapitation after 24 hours of CCl_4 injection.

Group IV (curative group): rats were injected i.p. with a single dose of 2% CCl_4 in vegetable oil (1 ml/kg body weight) then after 24 hours they were treated orally with 200 mg L-arginine in (1 ml tween/kg body weight) daily for six days and were sacrificed by decapitation.

Samples

After decapitation of rats of each group, their livers were rapidly rinsed with ice-cold saline, dried on filter paper, then homogenized in ice-cold distilled water and were used for biochemical analyses.

Biochemical analyses

Estimation of glutathione (GSH) content was performed spectrophotometrically at 412 nm, using Elman, s reagent[16]. Glutathione peroxidase (GPx) activity in tissues was assayed by Little and

O'Brien method[17]. The activity of glutathione reductase (GR) was estimated using Beutler method[18]. Glutathione S-transferase (GST) activity was determined by the method of Habig *et al.*[19]. SOD activity was measured by the adopted method of Misra and Fridovich[20]. The activity of catalase was kinetically determined by monitoring the rate of decomposition of hydrogen peroxide (H_2O_2) as substrate using Chance and Mackley method[21]. Malondialdehyde (MDA) was estimated using its ability to react with thiobarbituric acid according to the method of Ohkawa *et al.*[22]. All biochemical assays, except for SOD, were expressed and referred to mg protein which will be determined in the liver tissues by the method of Bradford[23].

Statistical analysis

Using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) results were expressed as mean±SD. A statistical analysis between two groups was performed using Student's unpaired t-test, and P value of <0.05 will be considered significant for all analysis.

RESULTS

Effects of L-arginine on Lipid Peroxidation and GSH

As shown in Table 1, evidence of lipid peroxidation (MDA) within the liver of CCl_4 - intoxicated rats was markedly increased as compared to the normal control group. Pre-treatment and post-treatment with L-arginine decreased the hepatic MDA content versus the untreated CCl_4– intoxicated rats. Furthermore, CCl_4-intoxication significantly decreased the level of hepatic GSH. Pre-treatment and post-treatment with L-arginine significantly increased the level of GSH compared to L-arginine untreated CCl_4 – intoxicated group.

Effects of L-arginine on Hepatic Antioxidant Enzymes

Table 2 shows that CCl_4 significantly inhibited hepatic activities of antioxidant enzymes (SOD, catalase, GPx, GR and GST) compared to the normal control group. Conversely, pre-treatment and post-treatment by L-arginine of CCl_4 –

Table 1: Hepatic contents of MDA and GSH

Animal groups	Control group	CCl_4 group	Protection group	Curative group
MDA(μM/mg protein)	0.509±0.069	2.747±0.307[a]	1.168±0.258[a b]	0.913±0.154[a b]
GSH(μM/mg protein)	5.790±0.556	4.589±0.375[a]	4.681±0.927[a]	7.805±0.458[a b]

All values are expressed as mean±SD.

a: P<0.001 versus the control group rats b: P<0.001 versus CCl_4 group of rats

Table 2: Activities of hepatic antioxidant enzymes

Animal groups	Control group	CCl_4 group	Protection group	Curative group
SOD(% inhibition)	49.310±6.016	25.644±1.246[a]	61.710±2.434[a c]	77.639±1.207[a c]
Catalase (μM/min/mg protein)	112.152±19.400	27.0820±9.983[a]	46.2993±12.001[a c]	56.2933±21.311[a c]
GPx(μM/min/mg protein)	5.389±0.453	3.947±0.501[a]	4.970±0.476[b c]	4.661±0.519[a d]
GR(μM/min/mg protein)	5.480±0.455	3.639±0.536[a]	4.759±0.438[a c]	4.462±0.517[a c]
GST(μM/min/mg protein)	5.667±0.432	3.314±0.534[a]	7.345±0.423[a c]	4.790±0.611[a c]

All values are expressed as mean±SD.

a: P<0.001 and b: P<0.01 versus the control group rats

c: P<0.001 and d: P<0.01 compared to CCl_4 group

intoxicated rats significantly enhanced the activities of hepatic antioxidant enzymes versus the corresponding enzymes of L-arginine untreated CCl_4 – intoxicated group of rats.

DISCUSSION

Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted[24]. GSH is a critical determinant of tissue susceptibility to oxidative damage and its depletion in liver tissue has been associated with an enhanced toxicity to chemicals such as CCl_4. The significant impairment of hepatic GSH and its associated with a substantial hepatocellular damage produced by CCl_4 suggested the determinant role of hepatic GSH in the development of CCl_4 toxicity[25]. In the present study and in accordance with Hewawasam et al. [26], a significant ($P<0.001$) decrease in the content of hepatic GSH was observed after injection of CCl_4 compared to the normal control rats. The pre- and post-treatment with L-arginine resulted in a significant ($P<0.001$) increase in the level of hepatic GSH, which might be due to the effect on de novo synthesis of GSH and its regulation or both. The level of hepatic GSH could be sufficiently maintained to counteract the progressive formation of free radicals in CCl_4 intoxication[25].

It is well known that, CCl4 causes liver damage by initiating the process of lipid peroxidation through the formation of lipid peroxyl radicals. Unless scavenged by free radical scavengers, these lipid peroxyl radicals abstract hydrogen atoms from lipid molecules, thereby propagating the process of lipid peroxidation[11]. Many compounds known to be useful against CCl_4-mediated liver injury, and they exert their protective effects either via decreased production of CCl_4 derived free radicals, or through their antioxidant activity[27].

Lipid peroxidation was significantly increased in the liver of CCl_4 intoxicated rats[28]. The present study showed that, the intoxication with CCl_4 caused extreme increase ($P<0.001$) of hepatic level of MDA supporting the previous observation of Zhu and Fung [29], who reported that the administration of CCl4 induced acute liver injury and significantly increased the level of TBARS (Thiobarbituric acid reactive substances; a byproduct of lipid peroxidation) in mice, in a manner it was both dose dependent and time dependent. Furthermore, our data agrees with the findings of Nanji *et al.*[30] who observed that animals with alcohol-induced liver injury treated with L-arginine had an approximately 50% decrease in the level of lipid peroxidation and about 50 to 60% decrease in the activity of cytochrome P450 2E1compared to the untreated ones. Conversely, Zhu and Fung[29] reported that L-arginine treatment had no significant effect on liver function of CCl_4-treated mice.

L-arginine has effective scavenger potentials in myocardial injuries; it can clear superoxide and possibly other reactive oxygen radicals[31]. Since, cytochrome P450 is a major contributor to lipid peroxidation in CCl_4-intoxication[10], nitric oxide (NO) generated from arginine reacts with cytochrome P450 causing the inhibition of its activity and the generation of free radicals [32]. The mechanism for the decreased lipid peroxidation by arginine administration might be related to NO ability, due to the presence of an unpaired electron, which could accept other electrons and function as scavenger, or it could be related to the antioxidant effects of L-arginine itself.

On the other hand, the obtained results in the present study revealed that CCl_4-intoxication significantly decreased the activities of hepatic antioxidant enzymes (SOD, catalase, GR, GST and GPx) as compared to the control group. Pre- and post-treatment with L-arginine significantly improved the activities of hepatic antioxidant enzymes compared to CCl_4-intoxicated rats. Oral supplementation of arginine in acute liver injury model significantly improved the state of liver injury[33, 34], implying that NO produced in experimental CCl_4 that treated with lipopolysacchariderats plays a protective role in the metabolism and removal of CCl_4. In another study using perfused rat livers, NO improved microcirculation and led to decreased hepatic damage in ethanol-induced hepatic injury[35], suggesting that NO plays a protective role in hepatic injury[36]. Low level NO acts as an antioxidant and higher level as a pro-oxidant. It was proposed that the mechanism of low concentration of $NO^{\cdot}$s protection may involve diminished metal-catalyzed lipid peroxidation and the high concentration of

NO's potentiating the oxidative stress may involve mitochondrial dysfunction[37]. Nitric oxide, besides, having antioxidant effects, it can also work as a pro-oxidant. Generally, when functioning as a nitric oxide donor in the treatment of inflammatory liver diseases the amount of arginine needs to be carefully titrated[28].

In conclusion, arginine administration decreases hepatic lipid peroxidation, induced by CCl_4-intoxication, and also enhances the activities of hepatic antioxidant enzymes (SOD, catalase, GPx, GR and GST). The mechanisms by which arginine exerts its protective and curative effect against CCl_4-injured liver might be through decreasing the production of CCl_4 derived free radicals, increasing the activities of antioxidant enzymes which in turn scavenge free radicals, or through the antioxidant effects of arginine itself or its derivatives. Finally, these results indicated that, arginine administration showed hepatoprotective and hepatocurative effects against CCl_4-induced liver damage and its curative effects were found to be more than the protective ones.

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