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# PROTECTIVE EFFECT OF AMINOGUANIDINE, A NITRIC OXIDE SYNTHASE INHIBITOR, AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN MICE

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## Summary

The present study was undertaken to evaluate the effect of aminoguanidine (AG) on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity. Treatment of mice with $CCl_4$ (20 µl/kg, i.p.) resulted in damage to centrilobular regions of the liver, increase in serum aminotransferase and rise in lipid peroxides level 24 hours after $CCl_4$ administration. Pretreatment of mice with AG (50 mg/kg, i.p.) 30 minutes before $CCl_4$ was found to protect mice from the $CCl_4$-induced hepatic toxicity. This protection was evident from the significant reduction in serum aminotransaferase, inhibition of lipid peroxidation and prevention of $CCl_4$-induced hepatic necrosis revealed by histopathology. Aminoguanidine, a relatively specific inhibitor of inducible nitric oxide synthase, did not inhibit the *in vitro* lipid peroxidation. Taken together, these data suggest a potential role of nitric oxide as an important mediator of $CCl_4$-induced hepatotoxicity.

*Key Words:* **aminoguanidine, nitric oxide, carbon tetrachloride, hepatotoxicity**

Carbon tetrachloride is a potent hepatotoxin producing centrilobular necrosis which cause liver injury. It has been widely accepted that liver injury produced by $CCl_4$ depends on its metabolism to highly reactive trichloromethyl ($CCl_3^{\cdot}$) radical which initiate lipid peroxidation (1,2). Others have suggested that active oxygen molecules such as superoxide radical may play an important role in the inflammation process after intoxication by $CCl_4$ (3,4).

Overproduction of nitric oxide in the liver has been implicated as an important event in endotoxin shock and in other models of hepatic inflammation and injury (5-8). Nitric oxide is known to react with superoxide radical, forming peroxynitrite, an even more potent oxidizng agent (9). Peroxynitrite can react directly with sulfhydryl residues in cell

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membranes leading to lipid peroxidation as well as with DNA resulting in cytotoxicity (10,11).

It has been reported that AG is a relatively selective inhibitor of an inducible form of nitric oxide synthase (12). Very recently it has been demonstrated that a single dose of AG (300 mg/kg) given 60 minutes before a toxic dose of cocaine, protected mice from cocaine-induced hepatotoxicity (13). Another study has shown that a single dose of AG (100 mg/kg) when administetred 30 minutes prior to acetaminophen, significantly decreased the liver necrogenic effects of the hepatotoxin in mice (14). Recently it has been shown that when AG (50 mg/kg) was administered to rats 30 minutes before a toxic dose of thioacetamide markedly diminished the severity of the liver injury (15). These results demonstrated that nitric oxide is an important mediator of hepatotoxicity. The present work was designed to examine the potential role of nitric oxide in the $CCl_4$-induced hepatotoxicity.

## Methods

Male Swiss albino mice (23-25 gram) were obtained from the Breeding Center, King Saud University, Riyadh, Saudi Arabia. The animals were housed at $22\pm1^0$C with a 12-hours light/dark cycle. The mice had free access to standard rodent food (Purina chow) and tap water *ad libitum*.

*In vivo studies*. Carbon tetrachloride (20 µl/kg, i.p.) was administered into mice in corn oil. Animals were injected with aminoguanidine (10, 25 and 50 mg/kg, i.p.) 30 minutes prior to $CCl_4$ administration. Aminoguanidine was dissolved in water by heating to approximately $60^0$C, followed by cooling at $37^0$C before administration.The control mice received corn oil or aminoguanidine.

*Biochemical estimations*. Serum aspartate aminotransferase (AST) was determined kinetically using commercial kit (BioSystems, Barcelona, Spain) (16). Thiobarbituric acid reactive substances (TBARS), measured as malonaldehyde (MDA), were determined in liver homogenates (17).

*In vitro studies*. Lipid peroxidation was carried out as described (18) with slight modification (19). The assay mixture contained varying amount of inhibitors (0.01-1000 µM in 10 µl volume), 0.75 ml phosphate buffer (50 mM, pH 7.4), 50 µl normal mice liver homogenate (10%) as a source of unsaturated lipids and 0.1 ml of 1 mM Ferric chloride. The contents were mixed thoroughly and peroxidation was initiated by 0.1 ml of 1 mM ascorbic acid. The tubes were placed at $37^0$C for 30 minutes and the extent of peroxidation was measured by TBA test. To the above tubes were added 0.1 ml BHT (2% w/v), 1.0 ml TBA (1% w/v in 0.05 M NaOH) and 1.0 ml TCA (2.8% w/v) and placed in water bath at $80^0$C for 20 minutes. At the end of incubation the tubes were cooled and centrifuged at 3200 rpm for 5 minutes. The chromogen was extracted with 2.0 ml n-butanol and absorbance was read at 532 nm. Tubes without inhibitors were subjected to TBA test under identical conditions and served as control.

*Histopathology*. Histological examination was performed on about 50% of randomized animals of each group. Liver samples were taken from the distal portion of the left lateral lobe. The tissue was fixed for at least 48 hours in 10% formalin. The samples were then embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin and eosin for examination by light micrography.

*Statistics.* All results expressed as mean ± SEM were analyzed using ANOVA followed by Dunnett's t-test. The acceptable level of significance was established at $p<0.05$.

## Results

*In vivo studies.* The present study was undertaken to evaluate the anti-hepatotoxic effect of aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride. The carbon tetrachloride injected into mice produced hepatotoxicity manifested as a significant rise in serum AST and lipid peroxides, measured as MDA, activity after 24 hours (Table I). Administration of aminoguanidine 30 minutes prior to $CCl_4$ injection showed marked inhibition of increased serum levels of AST and significant protection of lipid peroxidation induced by the hepatotoxin.

**TABLE I**

Effects of Aminoguanidine on Serum Activity of Aspartate Aminotransferase and Hepatic Lipid Peroxides in Mice Intoxicated with Carbon Tetrachloride

| Treatment | Hepatic MDA ηmol/g wet tissue | AST IU/L |
|---|---|---|
| Control (corn oil) | 169 ± 11.2 | 148 ± 10.4 |
| AG (50 mg/kg) | 171 ± 10.6 | 139 ± 10.9 |
| $CCl_4$ (20 µl/kg) | 396 ± 78.6* | 1297 ± 129** |
| $CCl_4$ (20 µl/kg) + AG (10 mg/kg) | 264 ± 21.0 | 356 ± 63# |
| $CCl_4$ (20 µl/kg) + AG (25 mg/kg) | 203 ± 17.8# | 261 ± 87# |
| $CCl_4$ (20 µl/kg) + AG (50 mg/kg) | 177 ± 32# | 251 ± 67# |

Mice were treated with aminoguanidine (10, 25 and 50 mg/kg, i.p.) 30 minutes prior to $CCl_4$ (20 µl/kg, i.p.) injections. Serum AST and hepatic MDA was determined 24 hours after $CCl_4$ administration. Values are expressed as means ± SEM for 9 to 10 mice and data were analyzed by one way ANOVA followed by Dunnetts' t-test. Significant increase ($p<0.001$**, $p<0.01$*) compared to control group. # Significant decrease ($p<0.01$) in comparision to $CCl_4$.

Pretreatment with aminoguanidine (50 mg/kg, i.p.) completely protected the liver against $CCl_4$-induced hepatic necrosis (Fig. 1A) and no signs of hepatic damage were observed. Mice treated with carbon tetrachloride (20 µl/kg, i.p.) showed extensive hepatocellular necrosis around the central vein after 24 hours (Fig. 1B). Control mice receiving aminoguanidine (50 mg/kg) or corn oil had normal hepatic architecture (Fig. not shown).

*In vitro studies.* To demonstrate that anti-hepatotoxic action of aminoguanidine is entirely different from compounds classified as antioxidants; we tested aminoguanidine as a possible inhibitor of hydroxyl radical mediated non-enzymatic lipid peroxidation in normal mice liver homogenate *in vitro*. Butylated hydroxytoluene (BHT) was used as

standard. Unlike BHT, aminoguanidine failed to show inhibition of lipid peroxidation upto tested concentrations (Fig. 2).

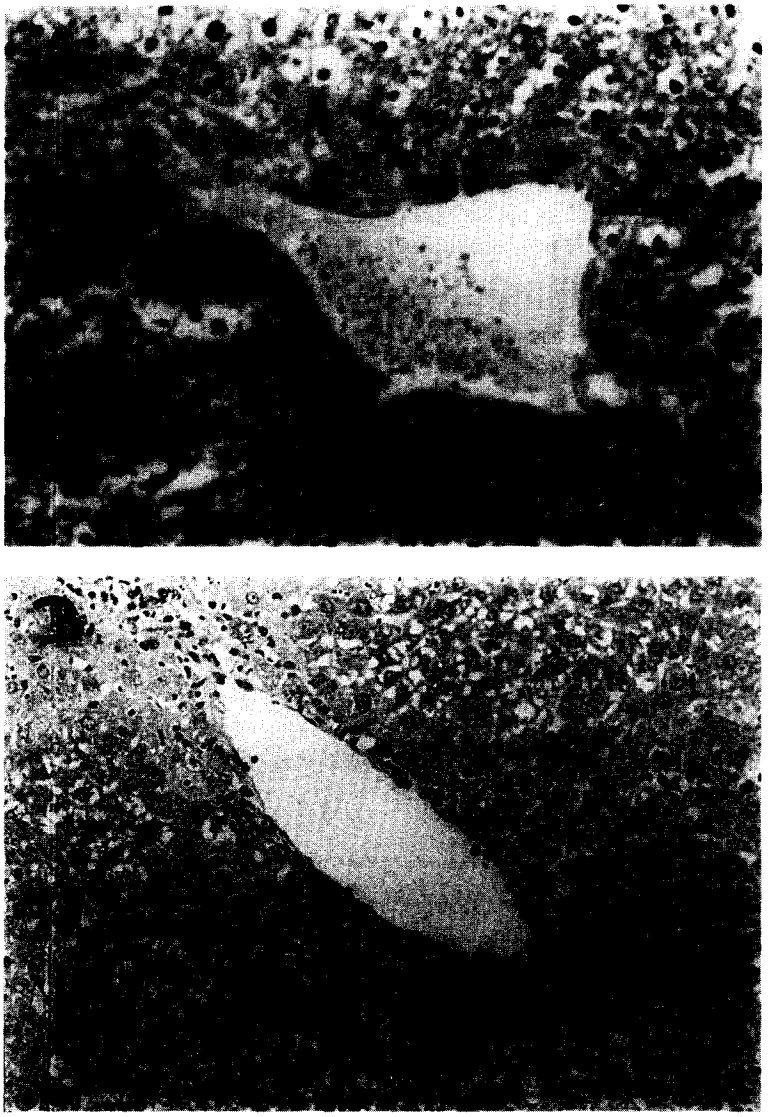

**Fig. 1**

Light micrographs of liver sections from mice. (**A**) mouse treated with $CCl_4$ (20 µl/kg, i.p.) and aminoguanidine (50 mg/kg, i.p.) showing a dilated central hepatic surrounded by normal hepatocytes. Note the absence of hepatocytes necrosis and the presence of mild autolytic/post-mortem cellular changes (hematoxylin and eosin X250). (**B**) mouse treated with $CCl_4$ (20 µl/kg, i.p.) showing extensive hepatocellular necrosis around the central hepatic vein. Note the absence of cell border and the disintegrated cytoplasm with nuclear and inflammatory debris. A rim of normal hepatocytes is seen in the peripheral parts of the photomicrograph (hematoxylin and eosin X100).

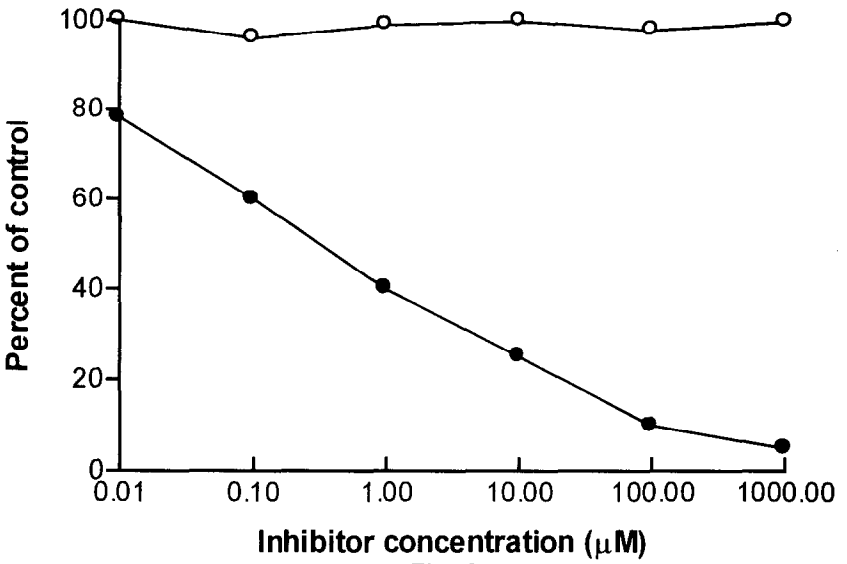


**Fig. 2**

Effect of aminoguanidine (open circle) and BHT (closed circle), a standard antioxidant, on the inhibition of non-enzymatic lipid peroxidation *in vitro*. Each point represents the mean of three independent but simultaneously carried out assays using three livers of normal mice (for clarity SEM bars are not shown; values were <10% of the mean).

## Discussion

Carbon tetrachloride-induced liver injury is a well established area of considerable toxicological interest. In the present study, the effect of single dose of aminoguanidine, a relatively selective inhibitor of inducible nitric oxide synthase (12) was used to evaluate the role of nitric oxide in the $CCl_4$-induced liver injury in order to gain further insights into the mechanism of its hepatotoxicity. Nitric oxide is a highly reactive oxidant produced by the liver parenchymal and non-parenchymal cells from L-arginine via nitric oxide synthase (20-22). There are numerous reports on the beneficial effects of aminoguanidine in various experimental models of inflammation and shock (13-15, 23).

The results obtained in the present study provide evidence that aminoguanidine, when administered prior to $CCl_4$, significantly decreases the liver necrogenic effects of the hepatotoxin. This beneficial effect of aminoguanidine preteatment could not be attributed to the direct inhibition of lipid peroxidation (Fig. 2). These data suggest that reactive nitrogen intermediates may contribute to tissue injury induced by this hepatotoxicant. It should also be noted that aminoguanidine not only act as inhibitor of nitric oxide synthase but also a peroxynitrite scavenger (24).

Alternative suggested mechanism of aminoguanidine protection could be by decreasing the metabolic activation of $CCl_4$ by directly inhibiting P4502E1, the isoenzyme most effective in the activation of $CCl_4$. However, it has been published recently that aminoguanidine upto 5 mmol/L had no effect on this isozyme *in vitro* (14).

In summary, the administration of aminoguanidine (50 mg/kg) 30 minutes before a toxic dose of $CCl_4$, protected mice from $CCl_4$-induced hepatotoxicity. The protective effect of aminoguanidine strongly suggest the involvement of nitric oxide in $CCl_4$-induced hepatotoxicity.

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