Magnesium attenuates carbon tetrachloride-induced hepatic injury in rats

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Abstract. In the present study, we investigated the protective effects of magnesium sulfate (MgSO₄) against carbon tetrachloride (CCl₄)-induced liver damage in rats. MgSO₄ (0.001, 0.01, 0.05 and 0.1 Mg²⁺ g/kg b.wt.) was administered intragastrically for 28 consecutive days to male, CCl₄-treated rats. The hepatoprotective activity was assessed using various biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT) and superoxide dismutase (SOD) activity. Histopathological changes in the liver, of different groups were also studied. Administration of CCl₄ increased serum ALT, AST, ALP and GGT, but decreased liver SOD activities in rats. Treatment with MgSO₄ significantly attenuated these changes to nearly normal levels. The animals treated with MgSO₄ showed decreased necrotic zones and reduced hepatocellular degeneration when compared to liver exposed to CCl₄ alone. Hepatic damage was reduced in MgSO₄-treated rats. Thus, our results suggest that MgSO₄ has potential for the treatment of liver damage resulting from chemical intoxication.

Key words: carbon tetrachloride, hepatoprotection, magnesium sulfate, rat

The physiological importance of magnesium (Mg²⁺), the second most abundant intracellular cation, is well recognized. Mg²⁺ plays a fundamental role in the structure, metabolism, and bioenergetics of the cell [1-3]. It has been shown to participate in over 300 enzymatic reactions in humans, particularly those involving energy utilization [4, 5]. Its involvement in glycolysis, cell respiration, and transmembrane transport of other cations such as sodium, potassium, and calcium has been reported [6]. Mg²⁺ is known to be involved in the control of metabolic processes such as glycolysis, nucleotide metabolism, protein synthesis, oxidative phosphorylation and glutathione-based detoxification processes. Regarding the latter, it has been shown that Mg²⁺ deficiency induces oxidative stress and increases tissue susceptibility to peroxidation in various organs such as heart, liver, skeletal muscle and testis [7-9]. Mg²⁺ may play a role in the immune response as a co-factor for immunoglobulin (Ig) synthesis, C3 convertase, immune cell adherence, antibody-dependent cytolyis, IgM lymphocyte binding, macrophage response to lymphokines, and T helper-β cell adherence [10]. Mg²⁺ deficiency contributes to an exaggerated response to immune stress, oxidative stress being a consequence of the inflammatory...
Hypomagnesemia has been associated with inflammation and increased production of free oxygen radicals. Because Mg$^{2+}$ acts as a natural calcium antagonist, the molecular basis for the inflammatory response may also be the result of a modulation of intracellular calcium concentrations [12].

The liver, a major site for metabolism and detoxification, is an organ that is highly exposed to orally-ingested drugs, and contains a high concentration of metabolizing enzymes. The liver plays a central role in the kinetics of absorption, distribution and elimination of most drugs. Therefore, most adverse drug reactions involve liver toxicity [13]. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or other, non-infectious agents [14]. The pharmacotherapeutic options available for liver diseases are very limited and there is great demand for the development of new, more effective drugs. The objective of this study was therefore to investigate the effects of magnesium sulfate (MgSO$_4$) in a carbon tetrachloride (CCl$_4$)-induced hepatotoxicity model in rats.

Materials and methods

Chemical

MgSO$_4$ was obtained from Sigma Chemicals (Poole, UK). CCl$_4$ was purchased from Merck, (Darmstadt, Germany). The kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and $\gamma$-glutamyltransferase (GGT) were purchased from Parsazmoon (Tehran, Iran). The kit for superoxide dismutase (SOD) was purchased from Randox (Crumlin, UK). All the other chemicals and reagents used were of analytical grade.

Animals

Adult male Wistar rats with body weights of 200-230 g were used in the study. The animals were maintained under standard environmental conditions (23-25°C, 12 h/12 h light/dark cycle) and had free access to standard rodent pellet diet and water. The normal diet contained the following (%): starch (40), casein (20), cellulose (6), sucrose (21), groundnut oil (2.5), corn oil (2.5), mineral mixture (7, including Mg$^{2+}$ 0.08%) and vitamin mixtures (1). The Mg$^{2+}$ concentration of the diet was 0.8 g/kg. Therefore, the rats were fed a 0.016 Mg$^{2+}$ g/kg b.wt. diet. The animals were acclimatized in the laboratory conditions for a week prior to the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985, no. 85-23). The Research and Ethics Committee of the College of Science, Islamic Azad University, approved the experimental protocol.

Experimental procedure

The animals were placed into 10 groups, with nine rats in each group. Treatment was then carried out as follows:

Group I received olive oil (intraperitoneally) and distilled water (intragastrically), and served as the untreated control animal group.

Groups II-V received MgSO$_4$ dissolved in distilled water daily via an intragastric tube (0.001, 0.01, 0.05 and 0.1 Mg$^{2+}$ g/kg b.wt.).

Group VI was the hepatotoxicity group that was given a suspension of CCl$_4$ (i.p., 0.5 mL/kg b.wt., 50% CCl$_4$ in olive oil), twice a week.

Groups VII-X were the treatment groups that received MgSO$_4$ dissolved in distilled water daily, via an intragastric tube (0.001, 0.01, 0.05 and 0.1 Mg$^{2+}$ g/kg b.wt.), with CCl$_4$ (i.p., 0.5 mL/kg b.wt., 50% CCl$_4$ in olive oil) twice a week.

After a 28-day treatment period, the animals were deprived of food overnight, anesthetized by exposure to diethyl ether, and then sacrificed by decapitation. Blood was collected from the jugular vein, and serum was separated and used for liver marker assays. Levels of ALT, AST, ALP and GGT were estimated using commercial kits [15-17]. The livers were dissected out, washed in ice-cold saline, patted dry, and weighed. The absolute and relative (organ-to-body weight ratio) weights of the liver were also measured for all rats when they were sacrificed. A small portion of the liver tissue was stored in 10% formalin for histopathological examination. From the remaining tissue, about 100 mg was weighed and homogenized in
phosphate buffer (pH 7.4) in a Potter–Elvehjem Teflon homogenizer. The homogenates were used for the assay of SOD [18].

Biochemical determinations
Blood samples from each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 3000 × g for 15 min. The enzyme activities of AST, ALT, ALP, and GGT in serum were evaluated spectrophotometrically, using commercially available diagnostic kits.

Preparation of hepatic homogenate
The weighed, frozen, liver tissue was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to obtain 1:9 (w/v) whole homogenate. The homogenates were then centrifuged at 11,000 × g for 15 min at 4°C and any cell debris was discarded. The supernatant was used for SOD assays [18].

Histopathological examination
Liver tissue samples from the experimental rats were fixed in 10% buffered formalin and were processed for paraffin sectioning. Sections of about 5 μm thickness were stained with hematoxylin and eosin for histological examination of the livers from all of the experimental rats. Liver sections were graded numerically to assess the degree of histological changes associated with hepatic injury. Centrilobular necrosis or zonal necrosis, which is characterized by damage to several liver cells around the central vein, fatty infiltration, and prominent ballooning, and bridging hepatic necrosis, a form of confluent necrosis of liver cells linking central veins to portal tracts or portal tracts to one another, were prominent in the histological findings [19]. The liver pathology was scored as described by French et al. [20], as follows:

- Score 0 = no visible cell damage.
- Score 1 = focal hepatocyte damage in less than 25% of the tissue.
- Score 2 = focal hepatocyte damage in 25-50% of the tissue.
- Score 3 = extensive, but focal, hepatocyte lesions.
- Score 4 = global hepatocyte necrosis.

The morphology of any lesion observed was classified and recorded [21].

Statistical analysis
All data were expressed as means ± S.E.M. Statistical analysis was carried out using one-way ANOVA followed by a Tukey post hoc test. The criterion for statistical significance was p < 0.05.

Results

Effect of MgSO4 on the body and liver weight of CCl4-treated rats
The results of the administration of MgSO4 on body and relative liver weights of rats in each group are shown in table 1. All rats survived the experimental period. Daily observations over the experimental period of 28 days showed no detectable alterations in the general states of the animals in any group. Body weights of the rats in CCl4-treated group had decreased significantly, but were found to be increased in control and MgSO4-treated groups. In contrast, relative liver weights were significantly increased in the CCl4-treated group. The administration of MgSO4 significantly reversed the CCl4-induced body and liver weights.

Effect of MgSO4 on biochemical parameters of CCl4-treated rats
The results for the hepatoprotective effects of MgSO4 on CCl4-treated rats are shown in figures 1-2. In the CCl4-treated control group, serum ALT, AST, ALP and GGT were significantly increased as compared with the untreated control group. In contrast, the groups that also received MgSO4 showed significantly less elevated levels of ALT, AST, ALP and GGT, and in a dose-dependent manner, compared to normal levels. Liver SOD activity in CCl4-treated rats was decreased significantly when compared with the control group. Treatment with MgSO4 protected this enzyme activity in a dose-dependent manner.

Histopathological evaluation
The results of hepatic histopathological examination are shown in table 2. When compared with
Table 1. Body weight, liver weight and weight gain of control or CCl4-treated (50% CCl4 in olive oil) rats with or without MgSO4 treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Body weight (g)</th>
<th>Final Body weight (g)</th>
<th>Weight gain (g)</th>
<th>Liver weight (g)</th>
<th>Liver weight/Body weight × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>200.13 ± 7.74</td>
<td>237.25 ± 7.30</td>
<td>37.12 ± 3.15</td>
<td>9.19 ± 0.22</td>
<td>3.87 ± 0.03</td>
</tr>
<tr>
<td>CCl4</td>
<td>201.14 ± 4.41</td>
<td>206.16 ± 9.36***</td>
<td>5.02 ± 1.13***</td>
<td>14.37 ± 0.14***</td>
<td>6.97 ± 0.05***</td>
</tr>
<tr>
<td>Mg2+ (g/kg b.wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>205.79 ± 8.63</td>
<td>239.48 ± 8.15</td>
<td>33.69 ± 5.82</td>
<td>9.78 ± 0.81</td>
<td>4.08 ± 0.03</td>
</tr>
<tr>
<td>0.01</td>
<td>202.30 ± 12.99</td>
<td>238.40 ± 4.79</td>
<td>36.1 ± 4.17</td>
<td>10.17 ± 0.32</td>
<td>4.26 ± 0.03</td>
</tr>
<tr>
<td>0.05</td>
<td>207.63 ± 7.51</td>
<td>231.07 ± 6.61</td>
<td>23.44 ± 1.09</td>
<td>9.59 ± 0.54</td>
<td>4.15 ± 0.04</td>
</tr>
<tr>
<td>0.1</td>
<td>205.24 ± 3.14</td>
<td>226.49 ± 5.12</td>
<td>21.25 ± 4.51</td>
<td>8.52 ± 0.73</td>
<td>3.76 ± 0.03</td>
</tr>
<tr>
<td>Mg2+ (g/kg b.wt.) + CCl4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001  + CCl4</td>
<td>208.87 ± 6.78</td>
<td>216.45 ± 5.14</td>
<td>7.58 ± 2.16</td>
<td>13.18 ± 0.33</td>
<td>6.08 ± 0.03</td>
</tr>
<tr>
<td>0.01   + CCl4</td>
<td>204.62 ± 4.62</td>
<td>226.16 ± 5.83 ++</td>
<td>21.54 ± 5.26 ++</td>
<td>12.82 ± 0.37 +</td>
<td>5.66 ± 0.02 +</td>
</tr>
<tr>
<td>0.05   + CCl4</td>
<td>203.12 ± 5.88</td>
<td>237.04 ± 5.62 +++</td>
<td>33.92 ± 6.17 +++</td>
<td>11.53 ± 0.27 +++</td>
<td>4.86 ± 0.03 +++</td>
</tr>
<tr>
<td>0.1 + CCl4</td>
<td>207.12 ± 5.59</td>
<td>242.31 ± 8.99 +++</td>
<td>35.19 ± 4.31 +++</td>
<td>11.38 ± 0.46 +++</td>
<td>4.69 ± 0.03 +++</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M for nine rats.

*** p < 0.001 significantly different from the control group.

+ p < 0.05 significantly different from the group treated with CCl4.

++ p < 0.01 significantly different from the group treated with CCl4.

+++ p < 0.001 significantly different from the group treated with CCl4.

the normal liver tissues from the vehicle controls, the liver tissue in rats treated with CCl4 revealed extensive injuries, characterized by moderate to severe hepatocellular degeneration and necrosis around the central vein, fatty changes, mononuclear inflammatory cell infiltration (lymphocytes infiltration), congestion, and sinusoidal dilatation (figure 3). However, the hepatic lesions induced by administration of CCl4 were remarkably less severe in rats had received concomitant MgSO4, and this was in a in a dose-dependent manner. The histopathological studies supported the hepatoprotective effect of MgSO4.

Discussion

The results of the present study demonstrated that MgSO4, given simultaneously with CCl4, effectively protected rats against CCl4-induced hepatotoxicity, as demonstrated by reduced AST, ALT, ALP and GGT activity measured in the serum, and increased SOD activity in the liver as compared to animals receiving CCl4 alone. This protective effect was also confirmed by histological observation.

In the present study, CCl4 treatment resulted in significant increases in the activity of AST, ALT, ALP and GGT compared to control rats. This result is consistent with the earlier report by Etim et al. [22]. CCl4, a selective, hepatotoxic chemical agent, is one of the toxins most widely used for the experimental induction of liver fibrosis in laboratory animals [23]. Liver injuries induced by CCl4 are the best-characterized models of xenobiotic-induced hepatotoxicity, and this approach is commonly used for screening the anti-hepatotoxic/hepatoprotective activity of drugs [24, 25]. In liver, CCl4 is metabolically activated by cytochrome P450-dependent mixed function oxidases in endoplasmic reticulum to form the CCl3 radical [26, 27]. The covalent binding of this radical to sulfhydryl-containing proteins in cells will initiate a chain of events leading to membrane lipid peroxidation and cell necrosis [28, 29]. In CCl4-treated rats many fold-increases in the activities of AST, ALT, ALP and GGT were observed. This can be attributed to the altered structural integrity of the hepatic cells.

When MgSO4 was administered simultaneously with CCl4 to rats, serum AST, ALT, ALP and GGT activities were found to be less elevated than in rats receiving CCl4 alone. MgSO4 protects the liver from the adverse effects of CCl4. Restoration of serum enzyme levels to normal levels in CCl4-treated rats after treatment with MgSO4 indicates the prevention of leakage of intracellular enzymes by stabilizing the hepatic cell membrane [30].
Magnesium and hepatic injury

Figure 1. Effect of oral administration of Mg$^{2+}$ at doses of 0.001, 0.01, 0.05 and 0.1 g/kg b.wt. on the activity of ALT (A), ALP (B), AST (C) and GGT (D) in serum from control or CCl$_4$-treated (50% CCl$_4$ in olive oil) rats. Each column represents mean ± S.E.M. for nine rats. Control group receiving olive oil (intraperitoneally) and distilled water (intragastrically). *** p<0.001 significantly different from the control group. + p<0.05, ++ p<0.01, +++ p<0.001 significantly different from the group treated with CCl$_4$.

In parallel with the alteration of liver function markers, these phenomena were also confirmed by histological observation. Severe and frequent degenerative and necrotic changes and the disrupted architecture of lobules, including fatty degeneration, the widespread infiltration of lymphocytes and extensive hepatocellular necrosis were common in CCl$_4$ intoxication. Rats treated concomitantly with MgSO$_4$ showed significantly fewer histological liver abnormalities including hydropic degeneration, dysplastic hepatocytes, bile-duct proliferation and perportal fibrosis, as compared with rats receiving CCl$_4$ only, highlighting the protective role of MgSO$_4$ in countering the hepatotoxicity of CCl$_4$. A distinct increase in perisinusoidal cells was observed histopathologically, probably indicating macrophage (Kupffer cell) activation.

Our results show that administration of Mg$^{2+}$ (at doses 0.01, 0.05 and 0.1 g/kg b.wt.) can attenuate CCl$_4$-induced hepatic injury in rats. These results are in agreement with other authors who concluded that pretreatment with Mg$^{2+}$ (0.02 g/kg b.w., 14 days) protected against cadmium-induced changes in GSH content in kidney and liver [31], and that oral Mg$^{2+}$ administration (0.05 g/kg b.w.) prevented cadmium-induced adverse effects on O$_2^·$ and malondialdehyde levels, and SOD activity in rat liver [32]. It is claimed that supplementation with Mg$^{2+}$ (0.04 g/kg b.w., four weeks) in cadmium-intoxicated rabbits caused a reduction in blood cadmium concentrations. Supplementation with Mg$^{2+}$ significantly decreased the cadmium concentration in the kidney, spleen, and bone [33]. Magnesium supplementation brings about a mild, positive influence on GSH
content following acute and subacute cadmium intoxication. It could be concluded that these findings contribute to the understanding of the cadmium/Mg\(^{2+}\) interaction, and suggest a positive role for Mg\(^{2+}\) in the treatment of cadmium poisoning. The doses of Mg\(^{2+}\) were chosen on the basis of literature data [33, 34].

It is reported that Mg\(^{2+}\) deficiency enhances reactive oxygen species (ROS) production and oxidative damage in chick embryonic hepatocytes [35] and endothelial cells [36]. The inflammatory response in Mg\(^{2+}\)-deficient rats suggests that Mg\(^{2+}\) deficiency might be accompanied by the activation of a number of cells including macrophages, neutrophils and endothelial cells [37, 38]. It is claimed that Mg\(^{2+}\) increases the permeability of the inner mitochondrial membrane, and decreases the coupling between oxidation and phosphorylation.

### Table 2. Histological injury score for livers with different doses of MgSO\(_4\) in control or CCl\(_4\)-treated (50% CCl\(_4\) in olive oil) rats.

<table>
<thead>
<tr>
<th>Index</th>
<th>Control</th>
<th>CCl(_4)</th>
<th>Mg(^{2+}) (g/kg b.wt.)</th>
<th>Mg(^{2+}) (g/kg b.wt.)+ CCl(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty degeneration</td>
<td>Cell swelling</td>
<td>Necrosis</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CCl(_4)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mg(^{2+}) (g/kg b.wt.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>0.01</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>0.05</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Mg(^{2+}) (g/kg b.wt.)+ CCl(_4)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0.001 + CCl(_4)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>0.01 + CCl(_4)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.05 + CCl(_4)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.1 + CCl(_4)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Magnesium and hepatic injury

[39, 40]. Therefore, by altering mitochondrial function, decreased magnesium could be involved in lipid peroxidation, cytokine induction, and induction of Fas ligands, well-known pathways for the development of steatohepatitis and fibrosis [41, 42]. Although enzymes involved in protection against oxidative stress are not known to be regulated by Mg2+, Mg2+ deficiency is known (1) to induce oxidative stress, (2) to increase susceptibility to peroxidation in various organs such as heart, liver, skeletal muscle and testes [7, 43, 44] and (3) to lower glutathione-peroxidase (GPX) activity [45, 46]. Conversely, Mg2+ supplementation was reported to decrease malondialdehyde (MDA) levels, MDA being a decomposition product of polyunsaturated fatty acid peroxides, and to increase SOD and GST activities in diabetic rats [47]. Mg2+ supplementation can reduce lipid peroxidation related to cadmium administration, and cadmium accumulation in organs [48]. Severe Mg2+ deficiency provokes pro-oxidative and pro-inflammatory changes [49-51], and also has been shown to be pro-apoptotic in liver, heart and thymus [52-54].
Figure 3. (continued)
Recent investigations suggest that Mg^{2+} is a potential therapeutic agent that may be used in the treatment of diseases linked with increased oxidative stress such as diabetes [47, 55] and asthma [56, 57]. Mg^{2+} has been reported to prevent the production of oxygen free radicals [58-60] and inhibit lipid peroxidation both in vitro [61-63] and in vivo conditions [64, 65]. Its deficiency has been shown to induce lipid peroxidation [8] and to produce a fall in the glutathione levels, superoxide dismutase, glutathione reductase and glutathione S transferase activity in red blood cells [66].

In summary, the results have shown that MgSO_{4} might have a hepatoprotective effect, thereby inhibiting the deleterious effects of free radicals generated by CCl_{4}. MgSO_{4} must be considered to be an excellent candidate for future studies on hepatic disorders.

Disclosure


References


Magnesium and hepatic injury


