The Protective Effect of Chitosan against Acute Oxidative Liver Injuries Induced by Carbon Tetrachloride

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Abstract. Carbon tetrachloride is an organic solvent known for its hepatotoxicity. Its effects are due to CCl₃ radical production. Chitosan is a natural antioxidant obtained from the exoskeleton of the crustacean; it protects the liver against the oxidative stress. We used 40 female Wistar rats (weight 230±25 gr.) divided into 4 equal groups. Group I was the control one; Group II received a unique dose of 3 ml/kg CCl₄ by gavage; Group III received daily 5 mg/kg vitamin E i.m. for a week before the CCl₄ administration. Group IV received daily 3 mg/kg chitosan i.p. for a week before the CCl₄ administration. 24 hours after the CCl₄ have been given, blood and liver tissue samples were taken. We assessed the oxidative stress markers (malondialdehyde) and antioxidant defence markers (hydrogen donors’ capacity and reduced glutathione) both from serum and liver tissue. CCl₄ acute administration induced oxidative stress especially in the liver tissue (malondialdehyde level increases, while glutathione level decreased). However, for the animals that have been protected with chitosan, the oxidative stress markers had almost similar values to those of the control group. The histopathological findings confirm the results. Therefore, chitosan has a protective effect against liver injuries induced by acute exposure to CCl₄.

Keywords: oxidative stress; chitosan; carbon tetrachloride; liver; vitamin E.

INTRODUCTION

The Reactive Oxygen Species (ROS) play an important role in the pathogenesis of the toxic hepatitis. In the liver tissue there are multiple ROS sources: the phagocyte membrane NADPH-oxidase; the xantin-oxidase system during the hepatic ischemia-reperfusion process and the microsomal enzymes (Mureşan, 1997).

Among the toxic substances metabolized by the liver are some organic solvents, the most important of them being carbon tetrachloride (CCl₄), quite frequently used in the industry and formerly used as an anaesthetic (Avram, 1994; Nenitescu, 1980).

Carbon tetrachloride – a lipid soluble substance – can pass through membrane of the hepatocytes. It is metabolized at the microsomal level where are specific p450 cytochroms. There the CCl₄ is transformed into CCl₃·, which attacks especially the lipids of the membranes (Chen et al., 2000; Sun et al., 2001; Croquet et al., 2003; Ichi et al., 2009).

Antioxidants’ supplementation generally has a positive effect on the oxidants/antioxidants equilibrium. Among the classic antioxidant substances, vitamin E is a free-radicals scavenger and neutralise the CCl₃· radicals, reducing thus the oxidative stress in
the hepatocytes (Farrell, 1994). Chitosan is a natural product obtained by the deacetylation of the chitin, which forms the exoskeleton the crustaceous. Its effect depends on the molecular weight (Suzuki et al., 1999; Tomida et al., 2009).

The aim of the present study was to assess the effect of the chitosan (a natural antioxidant substance) supplementation in comparison with that of the vitamin E (a classic antioxidant) on the oxidant/antioxidant equilibrium in the acute hepatitis induced by CCl₄ administration.

MATERIALS AND METHODS

We used 40 female Wistar rats (Rattus Norvegicus, strain Wistar), weight 230±20 gr., divided into four equal groups. They were kept room temperature (23±2°C), with 12 hours light-dark cycle, food and water ad libitum. They were allowed to acclimate for 1 week prior to experimental procedures.

Group I (n=10) was the control group; blood was obtained from the retroorbitar sinus and then liver tissue samples have been taken. The rats in group II (n=10) received a unique dose of 3 ml/kg of CCl₄ through gavage. The animals from group III (n=10) received i.m. daily, for a week, 5 mg/kg vitamin E; in the seventh day they received a unique dose of 3 ml/kg of CCl₄ through gavage. The animals from group IV (n=10) received i.p. daily, for a week, 3 mg/kg chitosan (Sigma-Aldrich®, medium molecular weight 190-310 kDa, acetylation degree 75-85%; the original solution contained 1 gr. chitosan/1000 ml acetic acid 0.1% brought, before use, to a neutral pH); in the seventh day they received a unique dose of 3 ml/kg of CCl₄ through gavage; 24 hours after the CCl₄ have been given, blood and liver tissues samples were taken.

We assessed from the blood serum and from the liver tissue markers of the oxidative stress, malondialdehyde (MDA) using the fluorimetric method (Conti et. al., 1991) and the markers of the antioxidant defence: hydrogen donors’ capacity (HDC), using Janaszewska method (2002) and reduced glutathione (GSH), using the fluorimetric method (Hu, 1994).

Also, we explored the histological changes using a Masson’s trichrome stain. The obtained data have been statistically analysed using SPSS 13.0.

RESULTS AND DISCUSSIONS

We assessed the descriptive statistics (mean, standard deviation, median, skewness and kurtosis). The mean values of the four experimental groups are presented in Tab. I and II. Since the distribution of the data was non-Gaussian, we compared the groups using non-parametric tests (Mann-Whitney). We considered significant differences at p<0.05.

<table>
<thead>
<tr>
<th>Lotul</th>
<th>MDA (nmol/ml)</th>
<th>HDC (% inhibiție)</th>
<th>GSH (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.25±0.30</td>
<td>34.43±4.17</td>
<td>5.61±0.64</td>
</tr>
<tr>
<td>II</td>
<td>1.85±0.34</td>
<td>34.44±6.18</td>
<td>27.92±14.48</td>
</tr>
<tr>
<td>III</td>
<td>1.74±0.26</td>
<td>33.97±4.08</td>
<td>14.82±3.04</td>
</tr>
<tr>
<td>IV</td>
<td>1.28±0.19</td>
<td>30.62±5.46</td>
<td>65.40±18.76</td>
</tr>
</tbody>
</table>

Tab. 1

Medium values of the oxidative stress markers in blood serum
Medium values of the oxidative stress markers in liver tissue

<table>
<thead>
<tr>
<th>Lotul</th>
<th>MDA (nmol/mg prot.)</th>
<th>HDC (% inhibiție)</th>
<th>GSH (µmol/mg prot.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.07±0.01</td>
<td>24.44±3.13</td>
<td>2.07±1.63</td>
</tr>
<tr>
<td>II</td>
<td>0.11±0.07</td>
<td>31.58±8.18</td>
<td>1.31±0.59</td>
</tr>
<tr>
<td>III</td>
<td>0.10±0.03</td>
<td>23.31±5.69</td>
<td>0.68±0.37</td>
</tr>
<tr>
<td>IV</td>
<td>0.09±0.02</td>
<td>25.34±3.19</td>
<td>0.94±0.74</td>
</tr>
</tbody>
</table>

The Mann-Whitney test demonstrated statistically significant differences between the malondialdehyde level of the four groups both in blood serum and in liver tissue. These results are illustrated in figures 1 and 2.

Fig. 1. The differences between total malondialdehyde serum levels

Fig. 4. The differences between liver tissue malondialdehyde levels

Acute carbon tetrachloride administration stimulates lipid peroxidation and increases total MDA level both in the serum and in the liver tissue. Both vitamin E and chitosan supplementation reduce only blood MDA levels, as compared with the CCl₄ group, but this effect is statistical significant only for the chitosan, which decrease the MDA to a level similar with that of the control group. Our results are consistent with those found in the literature. Yu et al. (2007) demonstrated that CCl₄ administration increases the ROS levels, leads to lipid peroxidation and to high MDA levels. Similar results have been found by Login et al. (2006) after chronic CCl₄ administration. Also, MacDonald-Wicks and Garg (2003) showed that vitamin E administration reduces the lipid peroxidation.

The differences between hydrogen donors’ capacity levels are show in figures 3 and 4, while those between the reduced glutathione levels can be observed in figures 5 and 6. Carbon tetrachloride increases the hydrogen donors’ capacity in the liver tissue, but not in the serum. Both vitamin E and chitosan are able to scavenge the free radicals produced by CCl₄, thus maintaining the HD capacity at levels similar to that of the control group. But the reduced glutathione levels have a different dynamics. CCl₄ administration decreases the tissue GSH level, while increasing the serum one. Therefore, after acute administration of the toxic only the liver GSH reserves are depleted. In the liver tissue, the effects of the vitamin E and chitosan supplementation on the GSH levels are similar to those on the HD capacity.
Our results are in agreement with those find in the literature. For instance, Yu (2007) shows that CCl₄ administration reduces the glutathione level in the liver tissue. Also, MacDonald-Wicks and Garg (2003) showed that vitamin E administration improves the antioxidant defence markers, protecting them. Anraku et al. (2009) showed that water soluble chitosan can be also used as a dietary supplement and, after four weeks of supplementation it increases the total plasma antioxidant activity and reduces the albumin carbonyl levels, but these effects are dose-dependent. Since the antioxidant properties of the chitosan can be observed in normal subjects, there are serious perspectives to use it as a supplement during different disease, among which in liver diseases. Also, its antioxidant capacity is similar, even superior, to that of the vitamin C. In another recent study, Anraku et al. (2008) demonstrated that the low molecular weight chitosan is a potent inhibitor of the ROS production, thus offering an antioxidant protection of the human serum albumin.

The histopathological examination did not identify changes in the liver tissue samples of the animals belonging to the control group (Fig. 7). For those receiving CCl₄ (group II), we observed vacuolar dystrophy in the centrolobular area, acute inflammatory infiltrate (with a large number of neutrophils) and many eosinophils. Also, we can see lipid dystrophy and hepatocytes necrosis in the perivenular area (Fig. 8). The animals protected with vitamin E (group III) had perivenular fatty and vacuolar dystrophy and inflammatory infiltrate with a large number of neutrophils (Fig. 9), but the lesions are less severe than those of the animals.
in group II. Finally, in the liver tissue of the animals protected with chitosan only mild lesions can be seen, with a few necrotic hepatocytes in the centrolobular area, a diffuse dilatation of the sinusoid capillaries, the inflammatory infiltrate being mild or even absent (Fig. 10).

The histopathological examination confirms the oxidative stress findings. CCl₄ induces severe liver lesions, while the chitosan protects against these oxidative injuries better than vitamin E.

**CONCLUSIONS**

1. Acute carbon tetrachloride administration induces lipid peroxidation and leads to oxidative stress (MDA increased in comparison with the control group).
2. Vitamin E supplementation reduces the oxidative stress both in blood serum and in liver tissue.
3. Chitosan supplementation induces a significant decrease of the oxidative stress to values similar to those of the control group.
4. The antioxidant effect of the chitosan on the lipid peroxidation is superior to that of the vitamin E.

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REFERENCES