Grape Seed Extract in Prevention and Treatment of Liver Toxic Cirrhosis in Rats

S. Buloyan, V. Mamikonyan, H. Hakobyan, H. Harutyunyan, H. Gasparyan

Abstract—The liver is the strongest regenerating organ of the organism, and even with 2/3 surgically removed, it can regenerate completely. Hence liver cirrhosis may only develop when the regenerating system is off.

We present the results of a comparative study of structural and functional characteristics of rat liver tissue under the conditions of toxic liver cirrhosis development, induced by carbon tetrachloride, and its prevention/treatment by natural compounds with antioxidant and immune stimulating action. Studies were made on Wister rats, weighing 120–140 g. Grape seeds extracts, separately and in combination with well-known anticirrhotic drug ursodeoxycholic acid (Urdoxa), have demonstrated effectiveness in prevention of liver cirrhosis development and its treatment.

Keywords—Carbon tetrachloride, GSE, liver cirrhosis, prevention, treatment.

I. INTRODUCTION

According to WHO, liver cirrhosis (LC) is the fourth common cause of death among people between 30 and 50, and the eighth most common killer overall.

The LC genesis is ascribed to chronic hepatitis, other inflammatory diseases, and toxins (alcohol, drugs etc.). Depending on its origin, the emphasis in treatment is on inflammatory diseases, and toxins (alcohol, drugs etc.).

Cirrhosis related fibrosis involves encapsulation or replacement of injured tissue by collagenous scar. It is the effect of perpetuation of normal wound healing response, leading to abnormal continuation of fibrogenesis. It progresses at variable rates depending on the etiology of liver disease, environmental and host factors.

Within recent years grape seeds and their extracts gained special attention as highly effective antioxidants.

The aim of this work is to study the anticirrhotic effect of grape seed extract (GSE) and its synergy with a well-known anticirrhotic drug, urso deoxycholic acid (Urdoxa), have demonstrated effectiveness in prevention of liver cirrhosis development and its treatment.

II. MATERIALS AND METHODS

A. Design of Animal Experiments

Studies were performed on Wistar rats, weighing 120–140 g. They were housed in a room at 22±2°C and 12 hours light/dark cycle and given food and water ad libitum. All animal experimental procedures performed according to Directive 2001/20/EC.

The animals were divided into 8 groups of six. The control group comprised 4 animals.

Group 1: Control group: intact animals

Group 2: CCl₄-intoxication (during 12 weeks)

The animals of groups 3–5 were treated long-term (8 weeks) with immunostimulating and antioxidant preparations after the LC model genesis lead by CCl₄-intoxication.

Group 3: Treatment with ursodeoxycholic acid after CCl₄-intoxication,

Group 4: Treatment with GSE after CCl₄-intoxication,

Group 5: Treatment with ursodeoxycholic acid and GSE after CCl₄-intoxication.

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Parallel to the intoxication process, during 12 weeks, animals of 6-8 groups were treated with immunostimulating and antioxidant preparations to prevent the genesis of LC.

Group 6: Treatment with ursodeoxycholic acid during CCl₄ intoxication,

Group 7: Treatment with GSE during CCl₄-intoxication,

Group 8: Treatment with ursodeoxycholic acid and GSE during CCl₄-intoxication.

B. Preparation of GSE and Ursodeoxycholic Acid

GSE is derived from the Vitis Vinifer Satira Hayreneq grape seeds, were given three times a week, through intragastric tube (gavage needle) at dose level of 200mg/kg.

Ursodeoxycholic acid was dissolved in olive oil and was administered three times a week through intragastric tube at dose level of 90mg/kg. [10].

C. Modeling of CCl₄ Intoxication

The toxic model of LC was induced by I/P injection of 1.0 ml/kg body weight of 10% CCl₄ in olive oil as a vehicle, three times a week for 12 weeks [11].

After which the animals were deprived of food overnight, anesthetized by Nembutal and then sacrificed. The liver tissue was dissected out and weighed. Tissue sample was taken from hepatic lobes and stored in 96° alcohol for histopathological examination. Samples were routinely embedded in paraffin blocks, sectioned at 4 µm and stained with Hematoxylin and Eosin for microscopic examination according to Van Gieson [12].

The samples were studied by GENEVAL light microscope with a CANON® digital camera, connected to a computer. Liver sections were graded numerically to assess the degree of histological features in acute hepatic injury. Hepatocyte necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of Kupffer cells and lymphocytes were prominent in histological findings [13]. The liver pathology was scored by Metavir scoring system

| Score 4 = global hepatocyte necrosis. [14], [15] |

III. RESULTS

A. Body and Liver Weight

Initial and final body weight, liver weight and relative liver weight of groups are presented in Table I.

<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>Relative Liver Weight (g liver/100 g body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control group</td>
<td>143.75±1.32</td>
<td>300.75±5.62</td>
<td>157±4.36</td>
<td>12.17±0.17</td>
<td>4.05±0.05</td>
</tr>
<tr>
<td>2. CCl₄-intoxication</td>
<td>140.67±3.74</td>
<td>250.33±5.12</td>
<td>109.67±4.47</td>
<td>14.09±0.56</td>
<td>5.63±0.18 ++</td>
</tr>
<tr>
<td>3. Treatment with ursodeoxycholic acid after CCl₄-intoxication</td>
<td>142.0±2.74</td>
<td>209.25±4.33</td>
<td>67.25±1.18</td>
<td>9.46±0.22</td>
<td>4.53±0.20 **</td>
</tr>
<tr>
<td>4. Treatment with GSE after CCl₄-intoxication</td>
<td>136.3±5.55</td>
<td>253.75±12.81</td>
<td>117.42±12.81</td>
<td>13.21±0.42</td>
<td>5.25±0.32</td>
</tr>
<tr>
<td>5. Treatment with ursodeoxycholic acid and GSE after CCl₄-intoxication</td>
<td>125.83±0.94</td>
<td>200.75±7.71</td>
<td>74.92±7.84</td>
<td>10.14±0.35</td>
<td>5.05±0.20 ns</td>
</tr>
<tr>
<td>6. Treatment with ursodeoxycholic acid during CCl₄-intoxication</td>
<td>138.25±4.03</td>
<td>193.25±3.12</td>
<td>55±3.11</td>
<td>8.63±0.66</td>
<td>4.46±0.28 **</td>
</tr>
<tr>
<td>7. Treatment with GSE during CCl₄-intoxication</td>
<td>115.33±12.38</td>
<td>197.67±12.99</td>
<td>76.67±1.76</td>
<td>11.09±0.10</td>
<td>5.61±0.39</td>
</tr>
</tbody>
</table>
| 8. Treatment with ursodeoxycholic acid and GSE during CCl₄-intoxication | 131.75±13.06 | 190.75±8.87 | 59±10.98 | 9.35±0.28 | 4.90±0.22 *

Values are mean ± S.E.M. of rats;

*** P<0.001 significantly different from the group of CCl₄-intoxication

** P<0.01 significantly different from the group of CCl₄-intoxication

* P<0.05 significantly different from the group of CCl₄-intoxication

Group 2 with CCl₄-intoxication demonstrated a significant decrease in body weight, compared with control rats. Also, in all groups treated with GSE and ursodeoxycholic acid, after and during CCl₄-intoxication, decrease in body weight was observed, compared with control group.

Liver weights and relative liver weight ratios were higher in CCl₄-intoxication group relative to control animals. In groups 4 and 7, treated with GSE, liver weight and relative liver weight was almost the same as in the control group. Groups 3, 5, 6 and 8, treated with ursodeoxycholic acid and GSE, show reduction in liver weight compared with CCl₄-intoxication and control groups.

B. Histopathological Observation

The severity of liver morphological changes and fibrosis during and after CCl₄ intoxication was scored and summarized in Table II.

The livers of rats from the control group had no noticeable histological changes (Fig. 1).

Severe histopathological changes were observed in CCl₄ intoxication group. There were extensive liver injuries,
characterized by fatty degeneration, necrosis and infiltration of inflammatory cells around the central vein and the portal tracts, severe hepatocellular degeneration, and congestion. Hepatocytes in the degenerative and necrotic regions had pyknotic nuclei. Pseudo lobules were formed actively and macrovesicular droplets were detected (Fig. 2).

Improvement was observed in the group treated with ursoodeoxycholic acid after CCl$_4$-intoxication. There was focal infiltration and lesion. The architecture of liver was almost recovered. Pseudo lobules were few, with slender fibrous bands and less salient than in the CCl$_4$-intoxication group (Fig. 3).

The group treated with GSE after CCl$_4$-intoxication demonstrated slow regeneration compared with the one treated with ursoodeoxycholic acid after CCl$_4$-intoxication. The histopathological hepatic lesions induced by the CCl$_4$-intoxication were remarkably improved by treatment with GSE. Necrosis was also observed in this group, as well as infiltration of inflammatory cells around the portal tracts, and focal hepatocellular fatty degeneration. However, the state of liver was much better than in the CCl$_4$-intoxication group (Fig. 4).

Recovery of liver architecture was observed in the group treated with ursoodeoxycholic acid and GSE after CCl$_4$-intoxication. Small focal lesions and karyolysis were observed. However, the architecture of lobules was almost recovered, pseudo lobules were few and subdivided into smaller lobules and the hepatocytes mostly had normal appearance (Fig. 5).

The stats of livers treated with ursoodeoxycholic acid and GSE during CCl$_4$-intoxication together and separately were relatively better, than groups treated with these drugs after CCl$_4$-intoxication, except for the group treated with ursoodeoxycholic acid after CCl$_4$-intoxication.

In the livers of group treated with ursoodeoxycholic acid during CCl$_4$-intoxication was observed focal necrosis and infiltration close to the portal tract, small lipid accumulation, granulation, karyolysis, the beginning of formation of fibrous bands and pseudo lobules. The state of the livers was much better in this group than in CCl$_4$-intoxication group, but not better than in the group treated with ursoodeoxycholic acid after CCl$_4$-intoxication (Fig. 6).

The group treated with GSE during CCl$_4$-intoxication developed moderate inflammation and necrosis, small amount of collagen formation around central veins and portal tracts. The state of the liver was better than in the group treated with GSE after CCl$_4$-intoxication and the group treated with ursoodeoxycholic acid during CCl$_4$-intoxication. GSE has demonstrated prevention effect in LC genesis (Fig. 7).

The group treated with ursoodeoxycholic acid and GSE during CCl$_4$-intoxication developed few cirrhotic changes, with small amount collagen formation around central veins and portal tracts. The architecture of liver was almost not disrupted, hepatocytes had mostly normal appearance. However, several instances of lipid accumulation in hepatocytes and focal necrosis were observed. The state of the liver was better than in the CCl$_4$-intoxication group (Fig. 8).
IV. CONCLUSION

This study demonstrated that GSEs possess low activity in the treatment of toxic liver cirrhosis after 12 week intoxication with CCl4, but during intoxication GSE displayed preventive effects in the genesis of liver cirrhosis. That is why GSE can be recommended for prophylaxis of liver cirrhosis. GSE demonstrated moderate synergies with the ursodeoxycholic acid. However, the study of GSE is to continue, the plan is to conduct a comparative study of the structural and functional characteristics of rat liver tissue and hematopoietic stem cells under the conditions of toxic LC development, and its prevention/treatment by natural compounds with antioxidant and immunostimulating action. Another objective would be to determine the GSE dosage that will have more expressed effect for prevention and treatment of LC.

REFERENCES