Histological Analysis of Protective Effect of Ascorbic Acid on Chromium Induced Hepatotoxicity in Rat

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SUMMARY
This study was done with the purpose of histologically assessing the protective effect if any of supplemental doses of vitamin C contributing towards chromium induced hepatotoxicity. This study involved 120 rats, which were randomly divided into 4 groups A, B, C, and D each having 30 animals. Each group was further sub-divided into 3 sub-groups, each having 10 animals according to the treatment time i.e. 2, 4, and 8 weeks. Group B animals were injected with Chromium (Cr) intra-peritoneally (i.p.). Control group A was injected with an equal volume of distilled water. Group C received oral Ascorbic acid (AsA) alone and group D received oral AsA and intra-peritoneal injection of chromium both. Animals of each sub-group were sacrificed 2, 4 and 8 weeks after treatment respectively. Chromium induced hepatotoxicity exhibited histologically as dilated central vein, congested vasculature hepatocellular necrosis and inflammatory response in hepatic tissue. Ascorbic acid pre-treatment showed similar but milder changes than comparable B group animals receiving chromium alone. Toxic effects of chromium were not totally abolished by AsA pre-treatment, but were reduced. Role of ascorbic acid as a detoxicant in chromium poisoning needs further trials and evaluation in terms of increased dosage, route of administration and time interval between ascorbic acid and chromium administration.

INTRODUCTION
Heavy metals polluting the environment are in abundance. Chromium (Cr) is one such element posing a serious threat to human health. Used extensively in industry, its hexavalent form (Cr VI) is known to be the most toxic and is considered in dealing with occupational health control. It's hepatotoxic, nephrotoxic, cardiotoxic, as well as hematological and pulmonary toxic effects are responsible for its increased mortality. Its large amounts are introduced into the environment through sewage water & sludge and effluent wastes from industries utilizing this metal, which are released mostly into natural water without being processed, thus contaminating it. Cr VI, absorbed easily through GIT and lungs, is rapidly transported and distributed to liver, kidney and blood. Hexavalent chromate is iso-structural with phosphates and sulphates, it is readily taken up by GIT and penetrates to many tissues and organs in the body. It is more toxic than other forms due to its strong oxidizing power and high transport rate through cell membrane. Intracellularly its subsequent reduction to Cr III accompanied by its oxidation properties with stable complex formation is important for toxic manifestations of the metal. High accumulation of Cr III in all tissues and organs is a strong indication of wide toxic potential of exposure to Cr VI in drinking water and ambient environment. It can be reduced by biological reductants like hydrogen peroxide and ascorbate. If sufficient Cr VI reaches target organ without being reduced, acute damage may occur. Extracellular Cr VI reduction seems to represent a detoxification mechanism.

Vitamins are capable of altering biological
effects of chromates. Ascorbic acid (AsA) or vitamin C, capable of reducing Cr VI effectively is known for its antioxidant and nutritional effect. Its supplemental intake is 500 mg/day. AsA supplementation can reduce Cr induced structural changes in plasma membrane of liver and kidney. A dose of 150 mg is effective as a reducing agent. In cases of vitamin C deficiency, chromates deposited on airways penetrated easily into epithelial cells followed by its intracellular reduction, which is believed to be a prerequisite for toxic manifestations of the metal. AsA pretreatment completely abolished chromium induced structural changes whereas post treatment 2 hours after intoxication had no protective role.

Another study reported abolition of cellular injury by AsA pretreatment. Aim should be to accelerate extracellular Cr VI reduction. In this regard reducing properties of AsA on Cr toxicity might prove beneficial.

AIMS AND OBJECTIVES

This study was done with a purpose of histologically assessing protective effect if any of supplemental doses of vitamin C contributing towards chromium induced hepatotoxicity.

MATERIALS & METHODS

One hundred and twenty male Sprague Dawley rats, mean weight 161 ± 13 gms were kept in animal house PGMI, Lahore. They were fed on commercial diet and water ad libitum. Care was taken regarding optimal light and temperature. The animals were given two weeks for acclimatization and then were randomly divided into four main groups A, B, C and D each comprising 30 rats.

Animals of group B received 1 mg / kg chromium intra-peritoneally (i.p.), as sodium dichromate (Na₂Cr₂O₇ - E. Merck), dissolved in 2 ml of 0.9% saline on alternate days. Group A animals received an equal volume of distilled water i.p., and served as control. The dose was adjusted on weekly basis according to the weight of the animal.

Group D received 1 mg/kg Na₂Cr₂O₇ of saline, i.p. on alternate days, and AsA 10 mg/kg dissolved in 2ml of saline, orally daily. First dose of AsA was given 24 hrs before the first dose of Na₂Cr₂O₇. After that AsA was given an hour before Cr administration. The dose was adjusted on weekly basis according to the weight of the animal.

Group C received an equal volume of AsA orally on daily basis.

Ten animals each from group A, B, C and D were sacrificed at 2, 4 and 8 weeks of treatment, 24 hrs following the last injection. This period was given to allow the excretion of the unbound Cr from the body.

Liver tissue fixed in 10% formalin, was embedded in paraffin Sections were cut at 4-5 μ and stained with H and E by standard procedure. Histological parameters were noted.

RESULTS

With Cr treatment alone, at 2 weeks the capsule was thickened and mild sinusoidal and central vein dilatation was observed (Fig 1). These changes progressed at 4 weeks. Cloudy swelling was seen in significantly enlarged hepatocytes. Focal areas of nuclear changes were also seen. Necrosis was graded ++ (Table 1). At 8 weeks, capsule thickened further. Positive signs of inflammation were observed in portal area. Biliary hyperplasia was there (Fig 2). Micro vesicular fatty change was observed in significantly enlarged hepatocytes and the necrotic changes advanced (graded +++). Inflammatory infiltrate invaded fairly large areas of hepatocytic necrosis. Kupffer cells were prominent (Fig 3). Occasional acidophilic bodies were also seen.

With AsA alone, no signs of inflammation were seen throughout the course of experiment. Hepatocellular architecture was preserved (Fig 4). There was slight dilatation of central vein which progressed to a significant value at 8 weeks.

In contrast, the combination treatment of Cr and AsA both, at 2 weeks showed slightly dilated sinusoids and central veins with mild micro vesicular fatty change at places. Necrosis was +. At 4 weeks, there was cloudy swelling of hepatocytes at a few places. Kupffer cells were prominent, but no inflammatory infiltrate was observed (Fig 5). At 8 weeks, there was thickening of liver capsule and engorgement of dilated central veins. Hepatocytic
Fig 1. A histological section of rat liver-Group B1 receiving chromium only for 2 weeks. 1. Central vein. 2. Dilated sinusoids. 3. Prominent kupffer cells. 4. Granular cytoplasm showing cloudy swelling. 5. Cells devoid of nuclei.

Fig 2. A histological section of rat liver showing portal tract-Group B3 receiving chromium only for 8 weeks. 1. Portal tract widening. 2. Biliary hyperplasia.

Fig 3. A histological section of rat liver-Group B3 receiving chromium only for 8 weeks. 1. Dilated central vein with distorted endothelium. 2. Hepatocytic cord devoid of nuclei. 3. Large necrotic Foci. 4. Prominent Kupffer cells.

Fig 4. A histological section of rat liver-Group C2 receiving Ascorbic acid alone for 4 weeks. 1. Central vein with few blood cells. 2. Hepatic laminae. 3. Sinusoids.

Table 1: Histological grading in the liver of a Sprague-dawley rat exposed to sodium dichromate (Chromium) and ascorbic acid alone and in combination for 2, 4 and 8 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control A (Weeks)</th>
<th>B Chromium administration for (Weeks)</th>
<th>C Ascorbic acid administration for (Weeks)</th>
<th>D Chromium and ascorbic acid administration for (Weeks)</th>
</tr>
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<tbody>
<tr>
<td>Histological grading</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>Normal architecture.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Mild to moderate congestion of central veins and sinusoids.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>Dilated central vein with distorted endothelium.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+++</td>
<td>Hepatocytic cord devoid of nuclei.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Normal architecture.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Mild to moderate congestion, minute areas of focal necrosis and prominent kupffer cells.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>Mild to moderate congestion, fairly large areas of focal necrosis, prominent kupffer cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+++</td>
<td>Capsular thickening, marked congestion, fairly large areas of focal necrosis, bile duct proliferation, prominent kupffer cells hyperplasia, inflammatory cells and widening around portal tract. Inflammatory cells in necrosed liver parenchyma.</td>
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degeneration and inflammatory cell invasion of portal tract was observed at few places (Fig 6). Necrosis was of lesser degree as compared to seen in animals receiving Cr alone for the same time period.

**DISCUSSION**

The minimum effective dose of Cr required to produce caustic effects is 1 mg/kg as used in this study. Cr induced hepatotoxicity became marked with advance in treatment, a finding consistent with reports by various authors. Cyanosis, a sequel of dichromate poisoning indicates increased oxygen extraction. Hypoxia results in impairment of Na pump mechanism leading to retention of Na and thus water. This in turn leads to cloudy swelling, hydropic degeneration, necrosis and thus nuclear changes. Impaired venous drainage leads to congestion, which in turn results in dilatation of blood vessels. Metabolic disturbances due to biochemical lesions could be exhibited as fatty change, cellular swelling and Hyaline degeneration followed by nuclear changes.

Inflammatory cells around portal tract are in response to necrosed hepatic tissue. Polymorphonuclear leucocytes and lymphocytes increased considerably to contribute their proteolytic enzymes to remove necrotic debris to aid the process of hydrolysis. Kupffer cells of macrophage system become prominent due to the same reason.

Biliary hyperplasia seen after 8 weeks, could be due to some obstruction in the biliary pathway as a response to chronic cell injury. Capsule thickening could be a result of increased collagen synthesis again due to chronic cell injury. Acidophilic bodies form as a consequence of hyaline degeneration leading to the formation of hyaline eosinophilic cytoplasmic bodies.

AsA alone showed no pathological results until 4 weeks. This was followed by mild to moderate congestion and dilatation of sinusoids, central veins and portal vessels. Daily requirement of AsA is 75-150mg, whereas median intake of supplemental AsA is 355-500 mg/day. Dose used in this study was median supplemental dose that could also cover for the antioxidant effect of AsA. No toxic effect of AsA has been reported as it is said to have high excretory ability.

In this study engorgement of blood vessels by blood cells seen after 8 weeks, needs further exploration. Animals getting both AsA and Cr at 2
weeks showed mild congestion and dilatation of central veins and sinusoids. Small areas of focal necrosis and kupffer cell prominence were noted. These effects progressed with advance in treatment and were almost the same as produced by Cr alone at this stage. So extra cellular Cr reduction was either incomplete or absent. Another study reported that 30 minutes AsA pretreatment before Cr administration resulted in complete abolishment of both metabolic disturbances and nephrotoxicity produced by Cr. The dose of AsA (500 mg/kg) used in this study was approximately 16 times the dose used by Na et al (30 mg/kg). The route of administration of AsA was intraperitonial, whereas in this study it was oral. In another study AsA by a mega dose of 5 g/kg prevented Cr induced protein urea, when as dose of 10 mg/kg of Cr was given. The reason for cellular reduction of Cr despite giving AsA, could be insufficient dose of AsA leading to incomplete or no extra cellular reduction of Cr. Route of administration of AsA could also have played a key role.

After 8 weeks of combination therapy of Cr and AsA, the degree of necrosis was much less than that of comparable group receiving Cr alone. There was no widening around portal tract. Kupffer cells were prominent meaning thereby the some but not all of the Cr got reduced extracellularly. The rest entered the cell and underwent intracellular reduction leading to Low-grade necrosis.

Role of AsA as detoxicant in chronic Cr poisoning regarding histological changes needs further exploration in terms of increased dosage, route of administration and interval between administration of the metal and AsA.

REFERENCES


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