Role of Ascorbic Acid in Portal Inflammation Induced by Rifampicin

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Abstract

Background: To assess the role of ascorbic acid in portal triaditis caused by Rifampicin.

Methods: In this experimental study, thirty adult BALB/c mice weighing 35-50 grams were taken. They were kept under standard laboratory conditions of temperature and humidity and light/dark cycle. Animals were given diet pellets. Mice were randomized and divided into three groups (n=10 animals in each group). Group A was given rifampicin in a dose of 100 mg/kg, Group B was given rifampicin 100 mg/kg along with Ascorbic acid 500 mg/kg orally for 6 weeks. Group C was the control group.

Results: Histological examination of animals revealed significant change in portal triaditis in experimental group having Ascorbic Acid along with Rifampicin.

Conclusion: Ascorbic acid has a protective role against Portal Inflammation caused by Rifampicin.

Key Words: Ascorbic Acid; Portal Inflammation; Rifampicin

Introduction

Tuberculosis is one of the major causes of morbidity and mortality among infectious diseases worldwide. About one third of world’s population has latent tuberculosis. According to a report published by World Health Organization Pakistan ranks 8th in terms of estimated number of cases with an incidence of 175/100,000 persons per year and a prevalence exceeding 1% of the total population.1-4

Isoniazid and rifampicin are the first line drugs for the treatment of tuberculosis. Damage to the liver caused by these drugs is a serious challenge in the course of treatment and creates difficulties in restarting the regimen. Toxic metabolites may play an important role in the development of drug induced hepatotoxicity due to antituberculous drugs. Rifampicin is a potent inducer of several cytochrome P450 isoenzymes leading to oxidative stress and production of reactive oxygen species (ROS) resulting in hepatotoxic injury. Rifampicin can cause hepatocellular injury resulting in portal triaditis.5-9

There is an increasing interest in the idea that certain diseases are associated with reactive oxygen species (e.g., oxygen free radicals, hydrogen peroxide). Cells have a number of mechanisms for dealing with the toxic effects of oxygen. Recent studies indicate the existence of a strong correlation between hepatic injury and oxidant stress in experimental animals treated with anti-tuberculous drugs. Studies have been performed to prevent or reduce the toxicity by the use of natural herbal drugs or synthetic compounds, without interfering with the therapeutic actions of the drugs. Garlic, Silymarin, N-acetylcysteine and several other herbal drugs are proved to have such effects.10,11

Ascorbic acid (AA) is a potent water soluble antioxidant. It has the capability of protecting cells from oxidative stress and exhibits anti-inflammatory properties by preventing increased production of cytokines. Studies have been performed which showed that vitamin C has protective effect on cell apoptosis. Treatment with ascorbic acid also arrests the activities of super oxide dismutase and glutathione peroxidase and inhibits the radiation-induced lipid peroxidation in the skin of mice exposed to different doses of fractionated gamma radiation.12,13

Materials and Methods

The study was carried out in the department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. Thirty adult male BALB/c mice having weight of 35-50 g and age between 10-12 weeks were obtained from NIH, Islamabad. The mice were kept in animal house under standard laboratory conditions in NIH. Animals were randomly divided into 3 groups each containing 10 mice. All mice were acclimatized for one week. These mice were maintained on pelleted diet prepared at animal house. They were kept at 12 hours light and dark cycle in a room at 22-24°C and were given food and water ad libitum.

Liver of the mice were put in 10% formalin in plastic containers for 48 hours. Filtered paraffin with melting point 56-58°C was used for embedding. Slides...
were prepared from right lobe of liver. Light microscopic examination was done after staining of slides with H&E. For portal inflammation slides were examined under 10X objective. It was graded as 0 if no portal tract showed inflammatory cells and 1 even if one portal tract was seen infiltrated by inflammatory cells.

The control group received regular laboratory diet for 6 consecutive weeks. The mice in the experimental group A were administered rifampicin orally in a dose of 100 mg/kg of body weight for 6 consecutive weeks. The mice in the experimental group B were administered rifampicin orally in a dose of 100 mg/kg of body weight with concurrent administration of ascorbic acid (analytical grade) in a dose of 500 mg/kg body weight for 6 consecutive weeks.

**Results**

Histological analysis of slides stained with H&E of control group C did not reveal portal inflammation in any member of group. Observation of experimental group A showed portal inflammation in 90% of animals (Fig 1) while in experimental group B only 50% of animals had portal inflammation while 50% of animals were normal (Fig 2). The findings were statistically significant when compared by applying Chi square test (Table 1).

**Table-1:** Comparison of Portal Inflammation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absent (Grade 0)</th>
<th>Present (Grade 1)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group C (n=10)</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Group A</td>
<td>10%</td>
<td>90%</td>
<td>0.00*</td>
</tr>
<tr>
<td>Experimental Group B</td>
<td>50%</td>
<td>50%</td>
<td></td>
</tr>
</tbody>
</table>

Statistical difference by applying Chi square in grading of Portal Inflammation between:
- Group A and B < 0.05*
- Group A and C < 0.05*
- Group B and C < 0.05*

**Discussion**

All cells of the body are exposed to oxidative stress. The oxidants causing stress come from exogenous and endogenous sources as a consequence of different metabolic processes. To combat these prooxidants, antioxidant defence system plays an important role. Fat and water soluble vitamins like alpha tocopherol, beta carotene and ascorbic acid contribute in providing antioxidant defence system of the body.14

Rifampicin is a part of antituberculous regime and is potentially hepatotoxic drug. It is metabolised and detoxified in liver making this organ susceptible to injury. Drug-induced hepatic injury is a major health problem and accounts for more than 50% of cases of acute liver failure.15

The idiosyncratic nature and poor prognosis of drug induced liver injury make this type of reaction a major safety issue during drug development. Mice, a commonly used species in medical research, were preferred due to their short gestation period and easy maintenance. Researchers have successfully used mice as a hepatotoxic model.16,17

To assess the protective role of antioxidants ascorbic acid was selected as it is easily available and has already received regulatory approval for treatment.18 There was remarkable portal inflammation in hepatic tissue of specimens obtained from group B animals. Presence of portal triaditis supports previous work which suggested that...
abundance of inflammatory cells are hallmark of hepatic tissue facing injurious impacts.\textsuperscript{19,20} Supplementation with ascorbic acid reduced inflammation as it is a potent antioxidant and reduces oxidative stress.\textsuperscript{21}

**Conclusion**

Ascorbic acid reduced the incidence of portal inflammation but did not offer complete protection as 50\% of animals still had portal inflammation.

**References**