Effect of Oral Ascorbic Acid Supplementation on Hepatotoxicity and Altered Immune Status in Alcoholics Without Overt Liver Disease

Moitrayee Chakrabarti 1), Ashim K Chakravarty 1), Asok Bandyopadhyay 2) and Asim Chakrabarti 3)

Abstract: The effect of large dose ascorbic acid supplementation on hepatotoxicity and altered immunological parameters in alcoholics without apparent liver disease was investigated. Supplementation restored serum gamma glutamyl transpeptidase (GGT) activity, serum albumin and serum immunoglobulin levels, T and B cell numbers in the peripheral circulation and ascorbic acid and dehydroascorbic acid levels both in plasma and leucocytes. This suggests an important role for ascorbic acid in combating free radical mediated damage and impaired immunological function in alcoholics.

Key words: chronic alcoholics, hepatotoxicity, immune status, mega ascorbic acid therapy

Introduction

Alcoholism is a major, worldwide health problem. The damage caused to hepatic cells by alcohol abuse can be attributed to alcoholic metabolites and alteration in immunological function 1). We have seen evidence of hepatic cell damage with concomitant changes in certain immunological parameters in alcoholics 6). We observed elevated serum GGT activity and decreased serum albumin levels in alcoholics, which is indicative of hepatocellular damage. The changes in immunological parameters included increased serum immunoglobulin levels and T cell numbers and decreased B cells in the peripheral circulation. Alcohol and its metabolites are hepatotoxic and the resulting damage possibly sensitizes T cells leading to further liver damage.

In this context we thought of using ascorbic acid as a potent detoxicant as it is known to counteract and neutralize the harmful effects of certain toxins in the body 7-10). Dietary ascorbic and also protects against many of the adverse effects of heavy metals 11-13) and nitrites 14). Ascorbic acid is not only a good reducing agent but is also an efficient radical scavenger of reactive ions 15).

Ascorbic acid is important for the functioning of various cell types including epithelial cells, polymorphonuclear neutrophils and macrophages 16-18). The frequency and severity of the common cold and the incidence of cancer are reduced by large doses of ascorbic acid 19-25). Several reports indicate that mega ascorbic acid therapy produces dramatic and...
rapid recovery in certain hepatic disorders\textsuperscript{26, 27}) but to date its effect on the immune system of alcoholics has not been elucidated.

In man on ingestion, ascorbic acid enters the body pool of ascorbic acid and the excess is efficiently excreted in the form of urinary ascorbic acid, dehydroascorbic acid and oxalate\textsuperscript{28}). The dehydroascorbic acid content of blood is negligible in normal subjects, but in certain infectious diseases dehydroascorbic acid can accumulate in blood and tissues, sometimes producing toxic effects\textsuperscript{29, 30}). As little is known about ascorbic acid and dehydroascorbic acid levels in alcoholics, we measured the level of ascorbic acid and dehydroascorbic acid in chronic alcoholics without overt liver disease during the course of mega ascorbic acid therapy.

In this study we investigated the possible protective effect of large doses of ascorbic acid on alcohol induced hepatotoxicity and altered immunological parameters in alcoholics without apparent liver disease.

Materials and Methods

Sixteen male habitual drinkers aged between 30–50 years, with similar socio-economic backgrounds and with a history of 5–15 years of alcohol abuse were chosen for the study. Due to the difficulty in obtaining chronic alcoholics without serious signs of liver damage, only sixteen subjects completed the study. Only those alcoholics were selected whose GGT values were higher than normal, thereby confirming hepatic damage\textsuperscript{6}). Sixteen normal controls of the same age group and similar socioeconomic background were used in the study.

After the initial tests, the subjects were treated orally with ascorbic acid at a dose of 3 grams per day, in three divided doses, for one month after which various parameters were measured according to standard protocols\textsuperscript{6}). Patients continued drinking during the whole period of ascorbic acid supplementation. Informed consent was obtained from each subject.

Quantitation of GGT activity in serum was carried out by Szasz method\textsuperscript{31}). Serum total protein was determined by the Biuret method and serum albumin by the dye binding method\textsuperscript{32}). Serum was fractionated for different types of proteins by standard electrophoretic methods\textsuperscript{32}). Immunoglobulins were measured by the immunodiffusion technique\textsuperscript{33}). The percentage of E. rosette forming cells was determined in reference to total lymphocytes following standard protocol\textsuperscript{34)} and with minor modifications as outlined earlier\textsuperscript{6}). The T and B cell ratio of peripheral blood was measured by the nylon wool fibre column separation technique\textsuperscript{35}). Plasma ascorbic acid and dehydroascorbic acid levels were determined by 2,6-dichlorophenol indophenol titration. The 2,4-dinitrophenyl hydrazine method was utilized for the determination of leucocyte ascorbic acid and dehydroascobic acid level\textsuperscript{36}).

The Students t Test was used to determine statistical significance\textsuperscript{37}). A p value of less than 0.05 was considered significant.

Results

GGT activity in serum: The initial GGT values in the alcoholic subjects ranged between 42–81 IU/l. Ascorbic acid supplementation reduced the elevated level of GGT in the alcoholics significantly (P<.025) towards normal values (Table 1). Interestingly the ascorbic acid therapy increased the serum albumin level towards normal from a mean value of 46\% to 52\% (Table 1). There was little change in the total protein level as a result of
Effect of Ascorbic Acid on the Immune Status of Alcoholics

Table 1. Changes in serum GGT activity, protein level and immunological parameters of alcoholics before and after ascorbic acid supplementation

<table>
<thead>
<tr>
<th>NO. OF PARAMETERS</th>
<th>NORMAL (MEAN±S.E.)</th>
<th>ALCOHOLICS BEFORE THERAPY (MEAN±S.E.)</th>
<th>ALCOHOLICS AFTER THERAPY (MEAN±S.E.)</th>
<th>SIGNIFICANCE OF DIFFERENCE (P) VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total Serum Protein in gm %</td>
<td>7.9 ± .5</td>
<td>7.7 ± .3</td>
<td>8.1 ± .18</td>
<td></td>
</tr>
<tr>
<td>2. Serum Albumin in gm %</td>
<td>4.5 ± .14</td>
<td>3.6 ± 1.1</td>
<td>4.2 ± .06</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>3. GGT in IU/l</td>
<td>11 ± 1</td>
<td>57.3±8.8</td>
<td>32.5±2.6</td>
<td>&lt;.025</td>
</tr>
<tr>
<td>4. % of Serum Albumin</td>
<td>58 ± .9</td>
<td>46.3±2.5</td>
<td>52 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>5. % of α1 Globulin</td>
<td>4.4 ± .5</td>
<td>5.4 ± .4</td>
<td>5.2 ± .6</td>
<td></td>
</tr>
<tr>
<td>6. % of α2 Globulin</td>
<td>8.5 ± .7</td>
<td>9.5 ± 1</td>
<td>9.0 ± 1</td>
<td></td>
</tr>
<tr>
<td>7. % of β Globulin</td>
<td>10.5 ± .3</td>
<td>15.5±1</td>
<td>12.8±1</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>8. % of γ Globulin</td>
<td>18. ± .9</td>
<td>23.2±1.9</td>
<td>21.4±2.1</td>
<td></td>
</tr>
<tr>
<td>9. IgG in mg/ml</td>
<td>11.4±1.2</td>
<td>22.3±2.9</td>
<td>27 ± 1.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>10. IgM in mg/ml</td>
<td>1.5 ± .2</td>
<td>3.3 ± .2</td>
<td>2.3 ± .3</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>11. % of B Cells</td>
<td>38.8±1.3</td>
<td>27.0±1.4</td>
<td>45 ± 4.4</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>12. % of T Cells</td>
<td>61.2±1.3</td>
<td>73 ± 1.5</td>
<td>55 ± 4.4</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>13. % of rosette forming Cells</td>
<td>57 ± .7</td>
<td>48 ± 2.9</td>
<td>49 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

*Only significant p values have been indicated

supplementation.

Electrophoretic analysis of serum proteins also showed an increase in the percentage of albumin after therapy. Other changes include a slight decrease in the percentage of both β and γ globulin (Table 1) in almost all cases indicating a slight reduction in the level of serum immunoglobulins. The mean values of α1 and α2 globulin in the alcoholics before therapy were 5.4% and 9.5% respectively, which remained almost unchanged after the ascorbic acid supplementation (Table 1). Quantitation of immunoglobulins carried out only in 3 patients showed a slight increase in serum IgG values and a simultaneous decrease in the IgM values in all cases (Table 1). The results indicate restoration of T and B cells numbers towards the normal range. However while there was a reduction in the percentage of T cells after therapy, the percentage of rosette forming cells did not change (Table 1).

The ascorbic acid level both in the plasma and leucocytes of alcoholics was lower than the normal controls but the dehydroascorbic acid level was elevated (Table 2). The reverse trend was observed after ascorbic acid supplementation for one month and the values both in plasma and leucocytes returned to the normal level.

Discussion

These results show that ascorbic acid and therapy of alcoholics partially restores the serum
Table 2. Plasma and leucocyte ascorbic acid and dehydroascorbic acid level in chronic alcoholics before and after ascorbic acid supplementation

<table>
<thead>
<tr>
<th>NO. OF PARAMETERS</th>
<th>PARAMETERS</th>
<th>NORMAL (MEAN±S.E.)</th>
<th>ALCOHOLICS BEFORE THERAPY (MEAN±S.E.)</th>
<th>ALCOHOLICS AFTER THERAPY (MEAN±S.E.)</th>
<th>SIGNIFICANCE OF DIFFERENCE (P VALUE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plasma Ascorbic Acid</td>
<td>1 ± .04</td>
<td>.65 ± .09</td>
<td>.82 ± .02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in mg/100ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Plasma Dehydroascorbic Acid</td>
<td>.07 ± .02</td>
<td>.36 ± .09</td>
<td>.14 ± .06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In mg/100ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Leucocyte Ascorbic Acid</td>
<td>26 ± 1.2</td>
<td>19.5 ± 4.5</td>
<td>24 ± 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>µg/10⁶ Leucocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Leucocyte Dehydroascorbic</td>
<td>0</td>
<td>27 ± 6.9</td>
<td>0</td>
<td>&lt;.005</td>
</tr>
<tr>
<td></td>
<td>Acid µg/10⁶ Leucocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only significant p values have been indicated.

GGT value and albumin level. This suggests that the therapy could prevent liver damage. Willis demonstrated the importance of ascorbic acid in maintaining healthy liver tissue free of cirrhotic and degenerative changes. It is known that liver cells are the main sites of synthesis of albumin. Simultaneously we observed an improvement in immune status with restoration of immunoglobulin level; T and B cell percentages in peripheral blood moved towards normal values (Table 1).

Increased numbers of activated T cells in alcoholics has been implicated in the early onset of cirrhotic changes previously. In the present study mega ascorbic acid therapy not only improved the damaged hepatocyte function, it also restored the percentage of peripheral T cells to normal levels.

Normally the level of blood ascorbic acid is higher than dehydroascorbic acid. In the alcoholic subjects the level of blood ascorbic acid decreased and simultaneously the level of dehydroascorvic acid increased (Table 2). Accumulation of dehydroascorbic acid in the blood of alcoholics might be a net effect of an increased rate of oxidation of ascorbic acid to dehydroascorbic acid and a decreased breakdown of dehydroascorbic acid. The oxidation of ascorbic acid when catalyzed by free cupric ion produces 1 mole of hydrogen peroxide per mole of dehydroascorbic acid. The toxic effects of H₂O₂ and free radicals are likely to affect different cell types including hepatocytes and immunoreactive cells.

Thus it seems that liver being the site of ascorbic acid metabolism has profound influence on the good health and functioning of different cell types including immunocompetent cells. Ascorbic acid supplementation is a promising therapy for the treatment of liver damage and impaired immunity in alcoholics.

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