Modulatory effect of quercetin on azathioprine induced membrane changes in the mouse spleen

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Received 12 November 1999; revised 1 November 2000

Modulatory effect of quercetin on azathioprine induced toxic changes was studied in spleen of experimental animals. Azathioprine treatment caused an increase in serum albumin/globin ratio and a decrease in total protein in spleen tissue. An increase in membrane bound ATPases was also noted. Supplementation of quercetin with azathioprine increased the protein content and lowered the activities of membrane ATPase in spleen. There was a decrease in serum albumin globulin ratio. It was concluded that quercetin modulated the protein and membrane bound ATPase activities and protected the spleen from azathioprine induced membrane damage.

Azathioprine (6,1-methyl-4-nitroimidazole-5-thio puine) is the main cytotoxic agent used for immunosuppression and is widely used to control tissue rejection in transplant surgery. After oral or intravenous administration, it disappears rapidly from the circulation and is extensively metabolised to mercaptopurine, purine analogue that inhibits DNA synthesis. Spleen is the most affected organ in the immunosuppressive condition. Other side effects associated with azathioprine include drug fever, liver damage and pancreatitis. Azathioprine exacerbates the condition and also causes joint and skin lesions. Other adverse effects are nausea, vomiting and pyrexia.

Flavonoids have been recognised as antioxidative and free radical scavenging agents and as membrane ATPase inhibitors. Quercetin [(3,3',4',5,7) pentahydroxy flavone] is one of the most common phenolic compounds in vascular plants. It occurs in conjugated or free form in many plant products used for food, including many fruits, vegetables and tea. In mice quercetin increases the hepatic and pulmonary activities. Increase in lung and liver activities may be either due to detoxification of the chemicals or by controlling the free radical mediated bioreactions. This confirms the antioxidant potential of quercetin. The present study was undertaken to study the modulatory changes in the spleen on co-administration of quercetin with azathioprine in an experimental mice.

Materials and Methods

Drugs and chemicals—Quercetin (commercially available powdered form was used) was obtained from M/s. Research Organics Chemical Company, Chennai, India. Azathioprine, bovine serum albumin were obtained from Sigma USA. Other chemicals used were of analytical grade.

Animals—Male albino mice (25 g) were maintained in the following standard environmental conditions. They were fed with commercial pelleted diet obtained from Hindustan Lever Limited and given food and water ad libitum. Animals were housed six per cage at 27±2°C with constant humidity under (55%) 12 hr light /dark cycle.

Quercetin in the powdered form was dissolved in dimethyl sulfoxide (0.8 mL) reagent and administered intraperitoneally (ip). Azathioprine was dissolved in water and was given orally (3mg/kg body wt) Animals were divided into four groups consisting of mice in each group, azathioprine tablets were administered orally, but experienced difficulty in administering DMSO by oral route.

Group I animals (control) received no treatment; group II animals received azathioprine (3 mg/kg body wt) for 28 days; group III animals received quercetin (25 mg/kg body wt) every week for 28 days (day 1,7,14,21 and 28); and group IV animals received simultaneously a treatment of azathioprine (3mg/l body wt) and quercetin (25 mg/kg body wt) for 7 days as given in groups II and III.

At the end of the treatment, the animals were fasted for 24 hr to avoid metabolic disturbances and sacrificed by cervical decapitation. Serum was collected by centrifugation for estimation of albumin globulin ratio. Agarose slide gel electrophoresis
serum protein in control and experimental animals was carried out. Spleen was dissected out, washed with chilled physiological saline, weighed, homogenised in 0.1 M Tris- HCl buffer (pH 7.4) at 4°C in Potter-Elvejem homogenizer and then used for evaluation of protein content and membrane bound ATPase activities. Activities of xanthine oxidase, adenosine deaminase and 5' nucleotidase were estimated in serum and spleen tissue.

Statistical evaluation — Student's t test and two way analysis of variance (Anova) were used for statistical analysis.

Results and Discussion
In azathioprine treated animals the albumin/globulin ratio increased as compared to control (Table I). No significant changes were noted in quercetin treated animals as compared to control animals. In group IV there was a decrease in albumin/globulin ratio as compared to group II animals. After azathioprine treatment, there was a depletion in γ-globulin levels as compared to control (Figs 1,2). In group IV animals, globulin level increased with a relative decrease in albumin/globulin ratio as compared to animals treated with azathioprine alone. In azathioprine treated animals, total protein also decreased as compared to control animals. However, in group IV mice there was an increase in total protein levels as compared to group II animals.

In group II animals membrane ATPase activities in spleen increased as compared to normal control (Table 2). Quercetin supplementation caused a decrease of membrane ATPases as compared to animals treated with azathioprine alone.

Group II animals showed an increased level of marker enzymes in serum with concomittant decrease in spleen tissue (Table 3). Quercetin treatment caused a decrease in serum enzyme levels and slight increase in tissue as compared to animals treated with azathioprine alone.

The results obtained indicated azathioprine induced toxicity in terms of increase in membrane ATPase activity, a known marker for membrane damage by azathioprine. Azathioprine has been reported to affect the functional activity of the spleen cells in vivo. Decrease in protein in azathioprine treated mice may be due to decrease in intra-mitochondrial ATP concentration. Mitochondrial membrane carrier for external ATP requires a transmembrane potential gradient which could be abolished by increase in mitochondrial Ca2+. Therefore sufficient ATP may not be available to drive mitochondria protein synthesis. Protein synthesis may be further inhibited in uncoupled mitochondria because of failure to import proteins. Since the processing of some

![Fig. 1](image-url) — Agarose slide gel electrophoresis of serum proteins of control and experimental animals [C— control; B — azathioprine treated; T — quercetin treated; and H— azathioprine and quercetin treated]

**Table 1** — Estimation of protein content and albumin and globulin ratio in the spleen and serum of experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Gr I)</th>
<th>Azathioprine (Gr II)</th>
<th>Quercetin (Gr III)</th>
<th>Azathioprine + quercetin (Gr IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>6.8</td>
<td>6.1**</td>
<td>6.9**</td>
<td>7.3**</td>
</tr>
<tr>
<td>(mg/g tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin globulin ratio</td>
<td>0.88</td>
<td>1.34**</td>
<td>0.90</td>
<td>0.92**</td>
</tr>
<tr>
<td>(percentage)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evaluation of significant variation in groups II and III are compared with group I; and group IV is compared with group II. Values are statistically significant when *P<0.05; **P<0.01; ***P<0.001.

Anova: table value of F for variation between samples and within samples at 0.5% level of significance = 0.253. The calculated value of F = 2.96 is more than the table value. So we may conclude that there is a significant difference within the groups.
cytoplasmically synthesised proteins depends on a transmembrane potential, uncoupling would prevent processing. Lack of cytoplasmic proteins could depress mitochondrial translation rates. Membrane bound Na+K+ ATPase is known as the sodium pump and is involved in pumping out Na+ cations in exchange for K+. This ATPase is active only in the presence of both Na+ and K+ ions.

Significant Mg2+ dependent ATPase activity is present in many tissues. However, its role in Mg2+ transport is not established. Mg2+ dependent hydrolysis of ATP may be coupled to a variety of cellular processes such as control of passive membrane permeability, regulation of oxidative phosphorylation in mitochondria and storage of catecholamines, serotonin, nucleotides and enzymes.

Increase in membrane ATPase and Ca2+ influx into cytoplasm is an indication of membrane damage. Borle has reported that Ca2+ ion sensitive ATPase located in the intestinal brush border may play a fundamental role in cellular uptake of calcium. It has been proposed by Birge et al. that this basal membrane Ca2+ ATPase mediates the energy dependent transport of calcium across the serosal membranes of intestinal cell. Increase in membrane ATPase activity was prevented by quercetin treatment. Fewell and Gromperts have reported that quercetin is an inhibitor of Ca2+ influx and exocytosis in rat peritoneal mast cells. Lang and Racker have reported the effects of quercetin in mitochondrial ATPase and energy linked reactions in sub-mitochondrial particles. Quercetin in group IV mice showed a decrease in ATPase levels which is in agreement with previous reports that quercetin is an inhibitor of membrane bound ATPase. It also confirms that quercetin interacts with Ca2+ and Mg2+ ATPase of sarcoplasmic reticulum as reported earlier. Kurikiy and Racker have further reported the

![Fig. 2](image-url) — Agarose slide gel electrophoresis of serum proteins of control and experimental animals.
inhibition of Na and K ATPase and its partial reactions by quercetin. Cytoprotective activity of quercetin could thus be ascribed due to their antioxidant property, and also to iron-chelating effectiveness. These findings have better prospects for the development and clinical application of this potent antioxidant.

Adenosine deaminase and 5′ nucleotidase are enzyme linked with acquired immunodeficiency. Adenosine deaminase converts adenosine to inosine in purine catabolism and has been reported as toxic to cells in culture. 5′ nucleotidase acts as a differentiation marker of B lymphocytes. During immune depression, levels of these enzymes are increased in the blood, due to spleen damage thereby causing a decrease in the tissue membrane. Xanthine oxidase converts xanthine to uric acid and increased level of xanthine oxidase and uric acid in the blood is an indication of membrane damage. Quercetin has been reported to prevent the decrease in xanthine dehydrogenase/oxidase ratio observed during ischemia reperfusion due to oxidative damage.

The results of the present study thus demonstrated that quercetin protected spleen from azathioprine induced damage by preventing peroxidation of membrane lipids. Further studies are however needed.

Table 2 — Activities of Na+K+ATPase, Ca2+-ATPase, Mg2+-ATPase and total ATPase in spleen homogenate of experimental animals

<table>
<thead>
<tr>
<th>Parameters (μmole of phosphorus liberated/hr. mg protein)</th>
<th>Control (Gr I)</th>
<th>Azathioprine (Gr II)</th>
<th>Quercetin (Gr III)</th>
<th>Azathioprine + quercetin (Gr IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+K+ATPase</td>
<td>0.48 ± 0.17</td>
<td>0.83 ± 0.1***</td>
<td>0.50 ± 0.1NS</td>
<td>0.43 ± 0.16***</td>
</tr>
<tr>
<td>Ca2+-ATPase</td>
<td>0.286 ± 0.3</td>
<td>0.43 ± 0.4**</td>
<td>0.26 ± 0.31NS</td>
<td>0.24 ± 0.19NS</td>
</tr>
<tr>
<td>Mg2+-ATPase</td>
<td>0.30 ± 0.1</td>
<td>0.68 ± 0.14***</td>
<td>0.31 ± 0.13NS</td>
<td>0.22 ± 0.11</td>
</tr>
</tbody>
</table>

Table 3 — Estimation of adenosine deaminase, xanthine oxidase and 5′ nucleotidase in the serum and spleen of experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Gr I)</th>
<th>Azathioprine (Gr II)</th>
<th>Quercetin (Gr III)</th>
<th>Azathioprine + quercetin (Gr IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase (μgptn/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>0.066 ± 0.08</td>
<td>0.052 ± 0.10***</td>
<td>0.06 ± 0.03</td>
<td>0.058 ± 0.08*</td>
</tr>
<tr>
<td>Serum</td>
<td>0.043 ± 0.76</td>
<td>0.075 ± 0.3***</td>
<td>0.04 ± 0.7</td>
<td>0.054 ± 0.52</td>
</tr>
<tr>
<td>Xanthine oxidase (μmole of uric acid formed/hr.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>0.201 ± 0.74</td>
<td>0.173 ± 0.54***</td>
<td>0.19 ± 0.79</td>
<td>0.180 ± 0.72*</td>
</tr>
<tr>
<td>Serum</td>
<td>0.165 ± 0.69</td>
<td>0.32 ± 0.3***</td>
<td>0.159 ± 0.66</td>
<td>0.210 ± 0.58**</td>
</tr>
<tr>
<td>5′ Nucleotidase (nmole/min/mgptn)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>22.4 ± 10.8</td>
<td>19.65 ± 8.5***</td>
<td>21.2 ± 12.1</td>
<td>20.15 ± 0.72*</td>
</tr>
<tr>
<td>Serum</td>
<td>19.2 ± 0.027</td>
<td>26.54 ± 0.026</td>
<td>18.25 ± 0.03</td>
<td>21.85 ± 0.028**</td>
</tr>
</tbody>
</table>

Evaluation of significant variation in groups II and III is compared with group I; and group IV is compared with group II. Values are statistically significant when *P<0.05; **<0.01; ***<0.001.

Anova: table value of F for variation between samples and within samples at 0.5% level of significance = 0.253. The calculated value of F = 0.352 is more than the table value. So we may conclude that there is a significant difference within the groups.
necessary to understand the mechanism of quercetin modulation azathioprine effect.

References
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