Effect of cyclophosphamide pretreatment on hematological indices of Indian Bonnet monkeys

Sir,

The leucocytes carried along with the transplanted tissue (passenger leucocytes) are important in graft rejection. It should be possible to greatly diminish the immunogenicity of a tissue graft if one can remove passenger leucocytes. Appropriate treatment of a tissue prior to transplantation can afford a marked reduction in its immunogenicity for the host. It is now possible to eliminate or at least reduce tissue immunogenicity by removal of leucocytes from the transplant prior to grafting. It therefore makes good theoretical and practical sense to attempt to alter tissue immunogenicity by treating the tissue to be grafted rather than the recipient.1

Cyclophosphamide belongs to the nitrogen mustard subclass of alkylating agents. It is an immunosuppressant that alkylates DNA, thereby interfering with its synthesis and function, particularly in proliferating (also in non-proliferating) lymphocytes. Both B and T cells are affected by cyclophosphamide, although the toxicity produced is greater on B cells than T cells. Consequently, this drug exerts its greatest effect by suppressing humoral immunity. When cyclophosphamide is administered in very large doses, it can result in a specific tolerance to any new antigen to which it is simultaneously exposed.2

Normal adult Indian Bonnet monkeys (Macaca radiata radiata) of both sexes, weighing between 2 and 6 kg were used in these experiments. After overnight fasting, about 1 ml of the blood was collected from saphenous vein and mixed thoroughly with the anticoagulant K3 EDTA. Cyclophosphamide (60 mg/kg) was administered intraperitoneally on day 0 and 2 after collecting a blood sample both the days. On day 4 the third blood sample was collected. The hematological indices such as hemoglobin, total leucocyte, differential leucocyte, platelet and reticulocyte were estimated. Hemoglobin was estimated by the Cyanmeth hemoglobin method with drabkins solution and reticulocyte were estimated. Hemoglobin was estimated as hemoglobin, total leucocyte, differential leucocyte, platelet and reticulocyte were estimated. Hemoglobin was estimated by the Cyanmeth hemoglobin method with drabkins solution and a photoelectric colorimeter. Total leucocyte count and platelet counts were done manually by the bulk dilution method using Turk’s and platelet diluting fluid (formal citrate) respectively. Reticulocyte count was done manually by a supravital staining at 37°C for 20 min with Brilliant cresyl blue dye. Blood smears made were stained by the Leishman’s stain and differential leucocyte count done. All the blood samples were also duplicated on an automated cell counter with five-part differential capacity (coulter MAXM) and a good correlation was demonstrated.

Totally, ten monkeys were used for this study. The statistical significance was determined using paired ‘t’ test and P<0.05 was considered significant. In Table 1, the change in the hemoglobin levels, platelet, reticulocyte and total leucocyte counts are shown. There was a slight but insignificant fall in the hemoglobin level and platelet count after two days and four days of treatment compared to pre-treatment values. There was no change in the reticulocyte count after two days of treatment but there was a significant fall four days after treatment.

Cyclophosphamide is expected to have more effect on the lymphoid tissues. To find out this effect, total leucocyte and differential leucocyte count were done. There was an insignificant reduction in the total leucocyte count two days after treatment: on the other hand there was a significant marked fall four days after treatment when compared to pre-treatment and two days post-treatment values. There was a significant increase in the percentage of neutrophils four days after treatment. There was no change in the percentage of lymphocytes two days after treatment, whereas there was a significant fall four days after treatment.

The percentage of and basophils had fallen two days after treatment, but came to the pretreated value four days after treatment. In the case of monocytes there was a slight increase after 2 days, but it came to the pretreated value four days after treatment (Table 2).

In rats cyclophosphamide has an immunotoxic effect on lymphocytes in the spleen and blood. It has also been reported that cyclophosphamide pretreatment in rats sharply decreased the activity of all lymphoid cells, especially the CD4+ lymphocytes.3 It was reported that both, destruction of donor antigen stimulated T cells in the periphery, and intrathymic clonal elimination of donor reactive T cells, were essential mechanisms of cyclophosphamide-induced tolerance.4

Table 1

<table>
<thead>
<tr>
<th>Blood samples</th>
<th>Hemoglobin g%</th>
<th>Platelet per mm²</th>
<th>Reticulocyte per mm²</th>
<th>Total Leucocyte per mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (before treatment)</td>
<td>12.4 ± 1.03</td>
<td>2.88,900 ± 113774</td>
<td>0.4 ± 0.59</td>
<td>10,720 ± 3469</td>
</tr>
<tr>
<td>Day 2</td>
<td>11.7 ± 1.18</td>
<td>2.64,700 ± 125778</td>
<td>0.4 ± 0.42</td>
<td>10,020 ± 3489</td>
</tr>
<tr>
<td>Day 4</td>
<td>11.6 ± 0.72</td>
<td>2.05,600 ± 9445</td>
<td>0.08 ± 0.09*</td>
<td>6,000 ± 5556*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 10 experiments. Statistical analysis between two days and four days after treatment compared with control. *P<0.01 compared to Day 2.
phosphamid pretreatment reduces the lymphocytes in the spleen and in blood. The reduction in the activity of the lymphoid tissue by cyclophosphamide could be the reason for a decrease in the lymphocytes in the peripheral blood of the monkeys.

Acknowledgements

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References


Histomorphological changes induced by Vitex negundo in albino rats

Sir,

Vitex negundo Linn. (VN) has been investigated extensively for its antiinflammatory and analgesic activities but it was only Tefang et al (1999) who noticed the inhibitory activity of the extract on prostaglandin biosynthesis and confirmed NSAID-like activity. But the type of cyclooxygenase inhibition produced by the extract is yet to be explored. Moreover, there are only a few studies regarding acute toxicity and very little is known about the histomorphological changes produced in various vital organs by toxic doses of VN. Therefore, the present study was undertaken to evaluate the histomorphological changes produced in various organs and to ascertain the type of cyclooxygenase inhibition produced.

The plant VN was collected from the local area of Sevagram, Dist. Wardha, Maharashtra and authenticated by an expert. The fresh leaves were shade-dried and powdered. The powder was macerated for 24 h in 70% v/v ethanol. Then it was subjected to percolation by using 70% v/v ethanol as solvent. The menstrum collected was again shade-dried and dissolved in distilled water to prepare an aqueous solution in the desired concentration just before use. Albino rats of either sex (weight 125-180 g) of Wistar strain were procured from the National Institute of Nutrition, Hyderabad. The study was cleared by the Ethics Committee constituted for the purpose. The animals were housed at 25 ± 2°C in polypropylene cages (4 per cage) with dust-free rice husk as bedding material and were provided with food and water ad libitum and were acclimatized for one week to laboratory conditions. The rats were fasted for 24 h before the experiment.

The acute toxicity study was carried out by administering VN leaf extract orally in graded doses (1-10 g/kg, body weight) to seven groups of animals each consisting of six animals. LD\textsubscript{50} of the extract was determined by graphical method and the histomorphological changes in vital organs were studied. The rats were observed continuously for two hours, then occasionally for a further four hours for any mortality onset and for the severity of any toxic sign. Finally, overnight mortality was recorded. The specimens of the stomach, liver, heart and lung from animals in which mortality was observed and of those which were sacrificed after 24 h were sent for histopathological examination to the department of pathology. The groups were compared using Z test and a \textit{P value} <0.05 was considered significant.

The results are shown in Table 1. The present study indicated that oral LD\textsubscript{50} dose of VN leaf extract is 7.58 g/kg, b.wt of rats. The LD\textsubscript{50} falls practically in the non-toxic dose range for a given compound as mentioned by Ghosh (1984). These findings are in agreement with those of Ravishankar et al. (1985). The stomach showed no histomorphological changes in any of the doses of the extract. These findings were contrary to the earlier study which proposed inhibition of prostaglandin biosynthesis. Prostaglandins are known to have cytoprotectant properties which help maintain the integrity of gastric mucosa, and NSAIDs with greater selectivity for COX-2 inhibition have lesser ulcerogenic potential. VN which is known to act by prostaglandin inhibition, may be expected to cause gastric damage but on the contrary it produced no histomorphological changes in the stomach even in toxic doses. This may be due to a selective COX-2 inhibition that might be responsible for the NSAID-like activity.

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (before treatment)</th>
<th>Post treatment Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil %</td>
<td>55.6 ± 16.3</td>
<td>53.2 ± 23.4</td>
<td>72.0 ± 12.2*</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>35.0 ± 15.2</td>
<td>34.7 ± 21.5</td>
<td>17.2 ± 9.8*</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>4.5 ± 3.4</td>
<td>2.9 ± 2.1</td>
<td>4.7 ± 3.5</td>
</tr>
<tr>
<td>Basophil %</td>
<td>1.5 ± 0.6</td>
<td>0.8 ± 0.5</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>3.2 ± 2.6</td>
<td>6.3 ± 8.8</td>
<td>3.5 ± 1.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 10 experiments. Statistical analysis between two days after treatment and four days after treatment compared with control. *\textit{P}<0.05 compared to Day 2