Immunotherapeutic Modification by *Tinospora cordifolia* of Abdominal Sepsis induced by Caecal Ligation in Rats

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Abstract

The protective effect of pretreatment with *Tinospora cordifolia* against mixed abdominal infection induced by caecal ligation was studied in rats of either sex, weighing from 150–200 g. They were divided into 4 groups: Group I received distilled water (control); Group II received metronidazole (200 mg/kg orally) and gentamicin (40 mg/kg IM) for 5 days after the caecal ligation was performed; Group III was given *Tinospora cordifolia* (100 mg/kg) orally for 15 days before and 5 days after surgery. Group IV received metronidazole, gentamicin and *Tinospora cordifolia* in the above doses. At operation a ligature was placed at the base of the caecum in each rat. The abdomen was re-opened in the surviving rats on the 5th post-operative day.

Pretreatment with *Tinospora cordifolia* reduced the mortality rate. This was comparable to that in the group given metronidazole and gentamicin. It also caused localisation of the infection and better scar formation. There was an increase in the peripheral neutrophil count and peritoneal macrophages, which was associated with increased phagocytic activity of macrophages.

Key words: Macrophage activation, abdominal sepsis, immunomodulator.

Introduction

Post-operative abdominal sepsis is a major cause of morbidity and mortality in spite of the availability of effective antimicrobial agents. The debate over the optimally effective chemotherapeutic regimen continues. Clinical studies evaluating the effectiveness of different approaches in the treatment of intraabdominal sepsis have yielded variable results.

An important factor causing a variable response to effective chemotherapy is the defence mechanism of the host. During the last decade, the importance of fortification of host defences has been realised and has led to the emergence of the concept of "prohost therapy" which aims at improving cellular immune functions. Currently, many synthetic immunological adjuvants and modulators are being investigated against microbial infection, with some success.

Ayurveda, the traditional system of Indian medicine, has several therapeutic regimens with plant products, which are claimed to fortify the host's defences against infection. We decided to see if some of these plants provided an advantage.

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The mortality and morbidity of the animals were recorded for 5 days following surgery. The surviving animals were sacrificed on the 3rd day using ether anesthesia. The abdominal cavity was opened after observing the external appearance of the operative wound. The ligated part of the caecum was inspected for the presence of pus, evidence of gangrene and for formation of a thick omental mass.

In Vitro Studies
The in vitro antimicrobial activity of *Tinospora cordifolia* was tested against various organisms. It was used in strengths ranging from 5 mg to 500 mg against *Bacillus subtilis* (1.5 × 10⁷ organisms/ml) and *Bacillus cereus* (3 × 10⁷ organisms/ml) grown on medium No 6, a group of animals received gentamicin in concentrations ranging from 0.1 to 7 mg/ml and served as control. *Tinospora cordifolia* was also tested against positive aures (4 × 10⁴ organisms/ml), *Pseudomonas aeruginosa* (1 × 10⁷ organisms/ml) and *Escherichia coli* (1 × 10⁶ organisms/ml) grown on nutrient agar with similar positive control of gentamicin.

The antibacterial activity of the sera obtained from rats treated with *Tinospora cordifolia* in doses of 100 mg/kg and 200 mg/kg was also tested against the same organisms. Large plate assays were carried out employing the method described by Reeves and Wisc.32

Statistical analysis
The leucocyte counts and percentage phagocytosis of macrophages were compared using Student's t test.

Results
The animals which did not receive any drug treatment (control group) died within 5 days after the surgery, while the mortality in Groups II (treated with metronidazole and gentamicin) and III (*Tinospora cordifolia* alone) was 33.3%. In the Group pretreated with *Tinospora cordifolia* and supplemented by treatment with metronidazole and gentamicin after surgery, the mortality was reduced to 16.6%.

| Table 1: Mortality rates and leucocyte and neutrophil counts in the treatment groups on various days |
|---|---|---|---|---|
| Groups | Mortality rate/day 5 post surgery | Leucocyte counts (mean ± SE) | Neutrophil counts (mean ± SE) |
| --- | --- | --- | --- | --- | --- |
| | | Day 0 | Day 15 of treatment (Day of surgery) | Day 5 post surgery | Day 0 | Day 15 of treatment (Day of surgery) | Day 5 post surgery |
| I (Control) | 6/6 | 960 ± 775 | 6850 ± 563 | — | 220 ± 332 | 1887 ± 549 | — |
| II (Metronidazole + Gentamicin) | 2/6 | 10100 ± 811 | 7450 ± 253 | 16270 ± 376 | 2648 ± 436 | 1831 ± 100.4 | 7833 ± 563 |
| III (*Tinospora cordifolia*) | 2/6 | 7950 ± 249 | 14030 ± 982** | 15750 ± 337 | 2203 ± 150 | 6854 ± 473** | 8521 ± 728 |
| IV (Metronidazole + Gentamicin + *Tinospora cordifolia*) | 1/6 | 5580 ± 1053 | 15000 ± 288** | 16400 ± 130 | 1152 ± 166 | 7820 ± 105** | 10123 ± 166 |

*p < 0.01, **p < 0.001 as compared to Day 0.

<p>| Table 2: Peritoneal fluid cell counts, percentage of macrophages and phagocytosis in the treatment groups on various days. |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Peritoneal fluid cell counts No of cells/ml (× 10⁹)</th>
<th>% macrophages</th>
<th>% phagocytosis of S aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 15 of treatment (Day of surgery)</td>
<td>Day 5 post surgery</td>
<td>Day 15 of treatment (Day of surgery)</td>
</tr>
<tr>
<td>I (Control)</td>
<td>0.573 ± 0.106</td>
<td>—</td>
<td>43.6 ± 4.5</td>
</tr>
<tr>
<td>II (Metronidazole + Gentamicin)</td>
<td>0.655 ± 0.26</td>
<td>5.83 ± 0.371</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>III (<em>Tinospora cordifolia</em>)</td>
<td>1.56 ± 0.15***</td>
<td>7.08 ± 0.411</td>
<td>66.5 ± 5.5***</td>
</tr>
<tr>
<td>IV (Metronidazole + Gentamicin + <em>Tinospora cordifolia</em>)</td>
<td>2.2 × 0.08**</td>
<td>7.94 × 0.433</td>
<td>66.5 ± 6.8***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 as compared to Day 0.
Autopsy examination in the control group revealed gangrenous, bloated caecum with collection of foul smelling peritoneal fluid in the abdominal cavity. In all the treated groups the site of operation appeared well sealed off, with dense adhesions forming an omental sac.

The results of the effect of the treatment with Tinospora cordifolia on peripheral white blood cells and peritoneal macrophages are summarised in Tables 1 and 2. Treatment with Tinospora cordifolia for 15 days produced a highly significant increase in the numbers of circulating white blood cells, predominantly neutrophils. The number of peritoneal fluid macrophages was significantly greater in animals treated with Tinospora cordifolia. Similarly, phagocytosis of S aureus by macrophages obtained from these treated animals was greater.

The peritoneal macrophages increased in number and showed activation five days after caecal ligation as expected in all the animals which survived. Those animals which received Tinospora cordifolia demonstrated a significantly higher number (p < 0.05) and greater phagocytic activity (p < 0.01) of macrophages when compared to those which received only metronidazole and gentamicin. There were no untreated control animals to compare at this stage as there were no survivors in that group.

Discussion
This study was conducted to evaluate the efficacy of Tinospora cordifolia against mixed abdominal infection induced by caecal ligation in rats. An attempt was also made to find out the possible mechanism of action. The efficacy of Tinospora cordifolia was compared with the chemotherapeutic agents commonly used to treat abdominal sepsis. The caecal tract harbours a large number of bacteria, but normally they are not able to invade the peritoneal cavity. In this experimental model, ligation of a part of the caecum created a blind pouch. This perhaps results in an overgrowth of bacteria which then invade the gut wall and gain access into the peritoneal cavity, producing sepsis. The onset process is controlled either by the establishment of an inflammatory barrier or by the destruction of the organisms by the host.

Pre-treatment with Tinospora cordifolia increased the survival rate in rats. This survival rate was comparable to that of the group treated with a combination of metronidazole and gentamicin, which was described as the most effective treatment against abdominal sepsis. Further, animals given a combination of Tinospora cordifolia and the anti-microbials had a lower mortality.

The efficacy of metronidazole and gentamicin combination in the prevention of sepsis is attributable to the bactericidal activity of the drugs. In our study Tinospora cordifolia was found to be devoid of any antibacterial activity. Further, the possibility of an antibiotic metabolite circulating in plasma was also ruled out by testing the antibacterial activity of the serum obtained from rats treated with Tinospora cordifolia, emphasizing the role of the immune mechanisms underlying its activity.

Evaluation of the effect of Tinospora cordifolia on polymorphonuclear cells and macrophage function revealed that pretreatment with Tinospora cordifolia increased both the peripheral leucocyte count and the phagocytic activity of the peritoneal macrophages. On the 5th post-operative day, the three treated groups showed an increase in peripheral neutrophils and peritoneal macrophages, with enhanced phagocytic activity. However, Groups III and IV which received pretreatment with Tinospora cordifolia showed significantly greater total leucocyte count, neutrophil count and phagocytic function of the macrophages as compared to the group which received only metronidazole and gentamicin.

Activated macrophages perform important functions in the arrest of infections. For example, they demonstrate increased phagocytosis. They also elaborate several secretary products; colony stimulating factor (or GM-CSF) is one such product which causes leukocytosis. The increase in leucocyte count also protects the animal against infection.

In view of the potency of drugs which increase non-specific resistance to microbial infections, the results of this study appear promising and warrant further examination of the clinical efficacy of Tinospora cordifolia in abdominal sepsis.

References