Immunotherapy with *Tinospora cordifolia*: A New Lead in the Management of Obstructive Jaundice

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Abstract

Objective: Immunosuppression associated with deranged hepatic function and sepsis results in poor surgical outcome in extrahepatic obstructive jaundice. The effect of an ayurvedic agent, *Tinospora cordifolia* (TC), which has been shown to have hepatoprotective and immunomodulatory properties in experimental studies, on surgical outcome in patients with malignant obstructive jaundice was evaluated.

Methods: Thirty patients were randomly divided into two groups, matched with respect to clinical features, impairment of hepatic function (as judged by liver function tests including antipyrine elimination) and immunosuppression (phagocytic and killing capacities of neutrophils). Group I received conventional management, i.e., vitamin K, antibiotics and biliary drainage; Group II received *Tinospora cordifolia* (10 mg/kg/day orally) in addition, during the period of biliary drainage.

Results: Hepatic function remained comparable in the two groups after drainage. However, the phagocytic and killing capacities of neutrophils normalized only in patients receiving *Tinospora cordifolia* (28.2±5.5% and 29.47±6.5% respectively). Post-drainage bactobilia was observed in 8 patients in Group I and 7 in Group II, but clinical evidence of septicemia was observed in 50% of patients in Group I as against none in Group II (p<0.01). Post-operative survival in Groups I and II was 46% and 92.4% respectively (p<0.01).

Conclusion: *Tinospora cordifolia* appears to improve surgical outcome by strengthening host defenses. (Indian J Gastroenterol 1993; 12: 5-8)

Key Words: Immunosuppression, neutrophil phagocytosis, neutrophil killing capacity.

Introduction

Despite improvements in perioperative care, outcome of surgery in patients with extrahepatic biliary obstruction has remained poor.1,2 Deranged hepatic function, bactobilia and compromised host defenses3,4 contribute to the high morbidity and mortality. Treatment of obstructive jaundice should therefore be aimed at improving liver function as well as towards enhancing host defenses. Present preoperative therapy aims only at decompressing the biliary tract and controlling infection with antibiotics. Stimulation of host defenses is a novel approach, whose use in the therapy for obstructive jaundice has not yet been explored.

Ayurveda, the traditional Indian system of Medicine, offers certain plant products termed as Rasayana which can strengthen the tissue resistance to disease.5 This claim appears to be akin to the 'prohost' therapy described in modern medicine.6 *Tinospora cordifolia*, a plant belonging to the Rasayana group of drugs, has been shown in experimental animals to provide protection against a variety of infections7,8 and to have a hepatoprotective effect.9 These experimental studies prompted us to evaluate the effect of *Tinospora cordifolia* in patients undergoing surgery for obstructive jaundice.

Methods

Thirty consecutive patients with obstructive jaundice (16 men, 14 women; age range 17-73 years, median 40 years) due to suspected malignant lesions attending our outpatient department between January 1987 and December 1989 were included in the study. The diagnosis of obstructive jaundice was based on clinical features, ultrasonography and percutaneous tranhepatic cholangiography, with final proof being obtained at a peroperative biopsy. All the biopsies confirmed the presence of malignant lesions; these involved the head of the pancreas (n=5), periampullary region (n=6), common bile duct and gall bladder (n=12) and mass in the porta hepatitis (n=7). Patients with inoperable or disseminated malignant lesions, those with other associated diseases, pregnant women and those taking other traditional drugs/medicines were excluded from the study. Written informed consent was obtained from all the patients. The study protocol
| Table: Hepatic and neutrophil function assessed at admission and prior to surgery |
|---------------------------------|----------------|----------------|
|                                 | Group I        | Group II       |
|                                | Basal          | Presurgery     |
|                                | Basal          | Presurgery     |
| Total bilirubin (μmol/L)        | 22.9±16.4      | 17.0±10.6      |
| Direct bilirubin (μmol/L)       | 15.2±10.1      | 11.7±7.9       |
| Total protein (g/L)             | 6.8±0.8        | 6.5±0.9        |
| Antipyrene half-life (h)        | 22.5±3.6       | 19.2±3.7       |
| Neutrophil phagocytosis (%)     | 21.2±3.7       | 19.6±5.1       |
| Neutrophil ICK (%)              | 22.9±4.4       | 17.7±7         |

Data are expressed as mean±SD; *p<0.01 in comparison with Group I and basal values

was approved by the institutional ethics committee.

At admission, liver function tests (plasma bilirubin, alkaline phosphatase, prothrombin time, total proteins and albumin, AST and ALT levels and antipyrine elimination test) were carried out. Ten milliliters of venous blood was collected in a sterile heparinized tube for determination of phagocytic and intracellular killing (ICK) capacities of neutrophils as previously described.12

Cell separation was done by buoyant density centrifugation.13 The neutrophil-RBC pellet thus obtained was mixed with 1 mL of dextran 70 and 1 mL of autologous plasma and allowed to sediment at 37°C. Supernatant containing neutrophils was separated and neutrophil count adjusted to 2-3×10⁶ cells/mL.

Assay for phagocytosis

To 0.1 mL of supernatant, 0.1 mL *Staphylococcus aureus* ATCC 6538 (adjusted to achieve a cell:bacteria ratio of 1:1) was added and volume made up to 1 mL with autologous plasma (in duplicate); after incubation at 37°C for 1 h, neutrophils were pelleted at 1000 rpm for 10 min. From one pellet, a Giemsa's stained smear was prepared, and the number of neutrophils containing intracellular organisms per 100 neutrophils was counted; this represented percentage phagocytosis.

Assay for ICK

Cells from the other pellet were washed three times with minimum essential medium (containing 100 μg/mL each of penicillin and streptomycin) and lysed using chilled, sterile distilled water so as to release the ingested organisms. Serial dilutions were made and number of viable bacteria counted by standard pour plate method on nutrient agar after 24 h incubation. Number of killed bacteria was calculated and expressed as percentage ICK.

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\% \text{ ICK} = \frac{\text{killed bacteria}}{\text{total phagocytosed bacteria}} \times 100
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The activity of neutrophils obtained from patients with obstructive jaundice was compared with those from 30 healthy blood donors (20 men, 10 women; age range 20-50 yr).

The patients were then randomly divided into two equal groups. Groups I and II were similar with respect to median age (42 and 40 yr respectively), sex distribution (M/F: 87 in both), average weight of patients (47±2 and 48.5±4 years) and median duration of illness (75 and 80 days). In Group I, distribution of malignant lesions was as follows: carcinoma head of pancreas – 2, perianpillary carcinoma – 3, carcinoma of common bile duct – 1, carcinoma gall bladder – 5 and mass in porta hepatis – 4; in Group II, these were 3, 4, 2 and 3 respectively.

Group I received conventional therapy including vitamin K, antibiotics (gentamicin and ampicillin) and percutaneous transhepatic cholangiography with drainage (PTCD). Patients in Group II received, in addition, a commercial formulation of *Tinospora cordifolia* (2 pills containing 65 mg each of dried aqueous extract of the stem) for 3 weeks after institution of biliary drainage. Dosage used (16 mg/kg/d in 3 divided doses orally) was based on previous animal experiments.59 Bile was collected for culture and antibiotic sensitivity testing at the time of insertion of drain, weekly thereafter for 3 weeks during drainage and intraoperatively. Antibiotic therapy was changed according to the sensitivity patterns, if necessary.

Biochemical and immunological investigations were repeated at the end of 3 weeks of drainage in both the groups following which biliary-enteric bypass surgeries, viz. choledochoduodenostomy (Group I = 7, Group II = 6), choledochojejunostomy Roux-en-Y (Group I = 3, Group II = 2), hepaticocoduodenostomy (Group I = 1, Group II = 3), hepaticocholedochojejunostomy Roux-en-Y (Group I = 1, Group II = 2), left hepaticocholedochojejunostomy segment III (Group I = 2, Group II = 1) and internal stenting (Group I = 2, Group II = 1) were performed. No patient underwent radical resection. Treatment with *Tinospora*
cordifolia was continued for 2 weeks post-operatively in Group II.

Patients were kept in hospital for 30 days after the surgical procedure. Deaths during this period were considered as postoperative deaths.

χ² test was used to compare post-operative mortality in the two groups. Student's t test for paired and unpaired data, with α-level of 0.05 was used for intra and intergroup comparisons of biochemical and neutrophil functions. Morbidity data (clinical sepsis) were compared using the Z test for proportions.

Results
At admission, both groups were well matched with respect to clinical features and hepatic and immune functions (Table). The neutrophil phagocytic and ICK capacities were significantly lower in patients with obstructive jaundice (20.3±4.5% phagocytosis and 19.6±5.8% ICK) than in 30 healthy donors (30.4±5.1% phagocytosis and 26.4±14.4% ICK; p<0.01 and p<0.05 respectively). Bile cultures at the time of PTC were sterile in all the patients. In Group I, bile cultures obtained at the end of 1, 2 and 3 weeks of drainage were positive in 1, 2, 2 and 3 patients respectively. In Group II these were positive in 2, 2, 1 and 2 patients respectively. In one patient bile became sterile during therapy with Tinospora cordifolia. Clinical evidence of sepsis was observed in 4 patients in Group I and none in Group II (Z=2.18; p<0.05).

After 3 weeks of biliary drainage, the hepatic functions remained comparable in the two groups (Table). Neutrophil phagocytosis (20.2±5.5%) and ICK (25.2±6.4%) however improved significantly in Group II as compared to the basal values (p<0.01), but not in Group I. The values in Group II prior to surgery were significantly higher than those in Group I (Table).

Of the 15 patients who underwent surgery in Group I, six (40%) survived. On the other hand, 14 patients (97%) in Group II survived (χ²=7.3; p<0.01).

The causes of death in patients in Group I were infection (n=5), liver cell failure (n=3) and hepatorenal syndrome (n=1) while the only death in Group II was due to sepsis.

Discussion
This study documents that therapy with an immunostimulant plant, Tinospora cordifolia, can improve the results of surgery in patients with extrahepatic malignant biliary obstruction.

Major operative risk factors in these patients include hepatic insufficiency and biliary sepsis; the latter often follows invasive diagnostic and preoperative biliary drainage procedures. Impairment of immune functions has been suggested as the underlying cause for sepsis, since these patients show reduction in delayed type hypersensitivity skin reactions and decreased clearance of micro-aggregated albumin by the hepatic reticuloendothelial system. In an earlier study, we found that the phagocytic and killing capacities of neutrophils in patients with obstructive jaundice were significantly depressed even before the onset of biliary infection. In an experimental model of cholestasis, these findings were confirmed and it was noticed that a primary defect in neutrophils and a factor present in serum (not yet characterized) contribute to immunosuppression. This increased the susceptibility of cholestatic rats to infection. These results corroborate the findings of Scott-Conner et al. and Roughan et al.

The potentiation of host defense mechanisms has not so far been considered in the therapeutic approach to obstructive jaundice. Our decision to include Tinospora cordifolia in the therapeutic regimen was based on our previous experimental study, which demonstrated that therapy with Tinospora cordifolia protected cholestatic rats against E coli induced mortality. In this model, this drug restored the normal activity of neutrophils and corrected the serum defect.

The present study has demonstrated the beneficial influence of Tinospora cordifolia on the surgical outcome (92% survival in treated patients vs 40% in those not receiving the drug). The causes of mortality in patients in Group I point to poor host defenses. Though the treatment had no significant influence on hepatic function, it did improve depressed neutrophil functions, thus substantiating the results of our previous animal experiments. It is also important to note that the incidence of bactoemia did not significantly differ in the two groups but colonization leading to sepsis was found only in patients not receiving Tinospora cordifolia.

In conclusion, strengthening of host defenses by Tinospora cordifolia appears to be responsible for improved surgical outcome in patients with malignant obstructive jaundice.

References
5. Rege NN, Narasimhan HM, Bapoo RD, Dahanukar SA. Modulation...