Anti-Cancer Drugs, 4, pp. 209–212

Immunomodulatory effects of a polysaccharide from *Tamarindus indica*

TT Sreelekha,[1] T Vijayakumar,[2] R Ankanthil,[1] KK Vijayan[3] and M Krishnan Nair[1]

[1]Regional Cancer Centre, Medical College, Thiruvananthapuram, Kerala 695 011, India. Tel: (+91) 0471 442541. Fax: (+91) 0471 437230
[2]The State Committee on Science, Technology & Environment, General Hospital Road, Thiruvananthapuram, Kerala 695 037, India
[3]Department of Chemistry, University of Calicut, Calicut University PO, Malappuram 673 635, India

A polysaccharide isolated and purified from *Tamarindus indica* shows immunomodulatory activities such as phagocytic enhancement, leukocyte migration inhibition and inhibition of cell proliferation. These properties suggest that this polysaccharide from *T. indica* may have some biological applications.

Key words: Acute lymphoblastic leukemia, leukocyte Migration Inhibition, phagocytosis, polysaccharide, *Tamarindus indica*.

Introduction

A number of plant polysaccharides have been studied in recent years in order to explore their potential as antitumor agents. The pods of *Tamarindus indica* is an essential ingredient of Indian dishes. The seeds are used as cattle feed and the leaves are used as a traditional medicine. The present paper deals with the isolation, purification and immunological properties of a polysaccharide from the seeds of *T. indica*.

Materials and methods

Isolation and purification

All procedures were done at 4°C. Crushed and dried seeds of *T. indica* (50 g) were defatted with petroleum ether (boiling point 60–80°C). The crude polysaccharide was extracted with water at 4°C. After filtration, ethanol (70%) was added to precipitate the polysaccharide. The crude polysaccharide was collected by centrifugation (20 000 *g*, 15 min), dissolved in water and dialyzed for 48 h against distilled water at 4°C. The supernatant was collected by centrifugation and shaken well with chloroform; the denatured protein in the form of gel was removed from the water–chloroform interphase.[1] The procedure was repeated until the water–chloroform interphase was clear. The aqueous layer was collected and dialyzed against distilled water and lyophilized.

Hydrolysis

The lyophilized sample (10 mg) was refluxed in 2 ml of 2N sulfuric acid in a water bath at 90°C for 3 h.[2] After filtration, the filtrate was freed from sulfate ions by precipitation with barium carbonate. Thin layer chromatography (TLC) was done on silica gel G (mesh 10–40 μm) with the solvent system acetonitrile:water, 85:15 (v/v). The constituent monosaccharides were detected with aniline diphenylamine/H_3PO_4.[3]

Procedure for biological assay

Phagocytosis. Venous blood (20 ml) was collected for each test in heparinized tubes. The blood was thoroughly mixed by gentle shaking and allowed to stand for 50 min at room temperature to sediment the erythrocytes. The supernatant rich in leukocytes was removed by pressing and washed twice with Hank's balanced salt solution. Neutrophils were counted in an aliquot after Geimsa staining. The cells were suspended in Hank's balanced salt

Financial assistance from the Council of Scientific and Industrial Research New Delhi is gratefully acknowledged.

Correspondence to TT Sreelekha

solution at a concentration of 2×10^{7} neutrophils/ml for the phagocytosis experiment.[2]

The reaction mixture consisted of 0.2 ml of neutrophils (2×10^{7}) in 1 ml of Hank's balanced salt solution containing 5% fetal calf serum and 0.05 ml of 10% sample. This mixture was preincubated in a water bath at 37°C for 7 min. A suspension of yeast particles was diluted with saline solution to 25×10^{7} particles/ml and heated at 100°C for 30 min. To the preincubated reaction mixture, 0.1 ml of the suspension of yeast particles was added and incubation continued at 37°C for 60 min. The number of yeast particles phagocytosed by neutrophils was counted after staining with 5% fuchsin in phenol solution. The influence of the polysaccharide from *T. indica* on phagocytosis was expressed as the phagocytosis index.

$$\text{phagocytosis index} = \frac{\text{no. of yeast phagocytosed by 100 neutrophils in test}}{\text{no. of yeast phagocytosed by neutrophils in control}}$$

The control was 0.1 M phosphate buffered saline (PBS, pH 7.2). The phagocytosis assay was done in blood run from 11 untreated acute lymphoblastic leukemia (ALL) patients and 11 normal healthy adults served as controls. The phagocytosis enhancement in ALL patients was compared with the controls by Student's *t*-test.

Leukocyte migration inhibition (LMI). Sterile precautions were taken while carrying out the experiment. Leukocytes were collected from normal controls, as in the case of phagocytosis, and prepared at a concentration of 1×10^{8} cells/ml. Then, 10 μl of leukocyte suspension was collected in 20 μl of capillary tubing by sucking with rubber tubing, and sealed with Plastecine ('seal-ease clay') at one end. The capillaries were centrifuged at 1000 *g* for 5 min and were cut at the cell–fluid interphase to separate the white layer of thrombocytes on the top. The cut capillaries were kept in migration chambers containing medium (TC 199) to avoid drying up. Then the migration chambers were wiped with alcohol, and silicon grease was applied over the rim of the chamber and also at one point inside the rim. Two micropets were inserted in each chamber with sealed ends in silicon grease at an angle of 40° so that the migrating area of the capillary did not intermingle. The chambers were filled with 50 μl of 10% polysaccharide diluted in medium.

The chambers were carefully mounted with coverslips to avoid trapping of air and formation of air bubbles. The chambers were incubated at 37°C for 18–24 h. These chambers were humidified to avoid drying up. After incubation the projected image of the migration field was drawn on paper using a camera lucida. The area of each migrated field was measured by planimetry.[4]

$$\text{calculation of migration indices} = \frac{\text{area of migration in test}}{\text{area of migration in control}}$$

Tissue culture medium (TC 199) was used as the control.

Inhibition of cell proliferation. Peripheral blood lymphocyte culture of 10 normal healthy individuals was carried out according to the standard procedure.[5] The polysaccharide was added at the time of initiation of the cultures and incubated for 72 h. The same experiment was repeated using unstimulated peripheral blood culture of seven ALL patients and incubated for 48 h.

Results

TLC analysis showed that the polysaccharide from *T. indica* consists of galactose, mannose and glucose. It showed immunomodulatory activities such as phagocytic enhancement, LMI and inhibition of cell proliferation. The phagocytic enhancement shown by the neutrophils collected from the ALL patients was much more than that in normal controls. The mean value obtained in the case of normal controls was 1.093 ± 0.014 (SD), whereas in ALL patients it was 1.297 ± 0.046 (SD); this difference is statistically significant ($p < 0.001$). The results are given in Table 1. Table 2 shows the migration indices and percentage of migration inhibition in the experiments. An average inhibition of 70–63% is shown by this polysaccharide.

Table 1. Effect of the polysaccharide from *T. indica* on phagocytosis

Phagocytic index	Normal control	Patient
<1	nil	nil
1–1.1	8	nil
1.1–1.2	3	nil
1.2–1.3	nil	5
>1.3	nil	6

Sample (5 mg) was dissolved in 1 ml of 0.1 M phosphate buffer at pH 7.2 and tested for phagocytosis. The phagocytic index of the control was calculated to be 1.000.

Table 2. Leukocyte migration indices and percentage of migration inhibition caused by the polysaccharide from *T. indica*

No.	Migration indices	Inhibition (%)
1	0.33	66.67
2	0.25	74.50
3	0.30	69.90
4	0.29	70.25
5	0.27	72.75
6	0.30	69.73
Mean	0.29	70.63
SD	0.02	1.99

Data were analyzed using Student's *t*-test to determine the significance. The values in the assay are mean ± SD of six samples. $p < 0.001$.

The addition of polysaccharide to lymphocyte cultures resulted in the arrest of cell division. When the polysaccharide was added at the time of initiation of the cultures there were no dividing cells at all, indicating that the polysaccharide has a blocking effect on the mitotic activity of phytohemagglutinin (PHA) stimulated lymphocytes. When the polysaccharide was added to the sample from ALL patients at the initiation of cultures, there was a marked reduction in the percentage of blasts, as indicated in Table 3.

Discussion

A number of plant polysaccharides isolated from various sources have been shown to exhibit antitumor activity.[6-8] Certain plant polysaccharides have also shown immunomodulatory activities.[9-11]

The immune system consists of a highly complex network of cells and molecules whose special characteristic is pattern recognition. The macrophage appears to be the first major cell type to react with an antigen in most immune responses. The macrophage has several types of surface receptors that enable it to carry out this function. There are non-specific membrane binding sites to which antigens may adhere; there are also surface receptors for immunoglobulin and receptors for the third component of complement (C3). The activity of complement depends on the operation of nine protein components acting in a sequence, which is referred to as the complement cascade. It is now recognized that this cascade can be activated by two pathways. The classical pathway is initiated by the antigen–antibody complex, to which complement then binds and assists in phagocytosis by the macrophage. The alternate pathway does not require specific antibody and can be activated at the C3 step by polysaccharides such as zymosan and the lipopolysaccharides.

It is suggested that the phagocytic enhancement obtained with the polysaccharide from *T. indica* may be due to the activation of complement (C3) and the initiation of complement cascade, i.e. the polysaccharide from *T. indica* may have the properties of zymosan or the lipopolysaccharides. They may activate the depressed functions of phagocytes in malignancies.

T lymphocytes serve a number of functions when activated by an antigen and the mechanisms of differentiation into those different subsets are unknown. Some T cells function as killer cells and are responsible for such cell-mediated immune responses as delayed hypersensitivity, allo-graft immunity and graft-versus-host responsiveness. Other T cells function as controls over the response of B cells and have a helper or suppressor role. There is as yet no consensus on the mechanisms by which these subsets of T cells exert their functions. The LMI may be due to the production of a lymphokine by sensitized lymphocytes in presence of the polysaccharide which affects the mobility of macrophages causing inhibition of the migration. This may be due to the antigenic nature of the polysaccharide or it is a cytotoxin.

A marked reduction in the proliferating capacity of lymphocytes of normal controls was observed when the polysaccharide was added at the time of the initiation of lymphocyte cultures. Repeated experiments employing peripheral blood of ALL

Table 3. Number of blast cells in the presence and absence of the polysaccharide in lymphocyte cultures

No.	Blast cells in the presence of the polysaccharide	Blast cells in the absence of the polysaccharide
1	13	93
2	32	87
3	17	91
4	12	96
5	14	89
6	21	78
7	19	94
Mean	18.28	89.71
SD	4.89	4.32

Data were analyzed using Student's *t*-test to determine the significance. The values in the assay are mean ± SD of seven samples. $p < 0.001$.

patients also revealed that this polysaccharide has a potential activity against proliferation of leukemic cells in cultures. These results suggest that the polysaccharide from *T. indica* also has antimitotic activity.

The phagocytic enhancement together with the LMI and the reduction in the cell proliferating capacity provide some biological evidence for antitumor activity of the polysaccharide from *T. indica*. Further detailed studies are necessary to elucidate this point. However, the above results suggest that the polysaccharide from *T. indica* may have potential biological applications.

References

1. Alam N, Gupta PC. Structure of a water-soluble polysaccharide from the seeds of *Cassia angustifolia*. *Planta Med* 1986; **50**: 308–10.
2. Yagi A, Nishimura N, Shida T, *et al.* Structure determination of polysaccharides in *Aloe arborescens* var. Natalensis. *Planta Med* 1986; **50**: 213–28.
3. Brautigam M, Franz G. Structural features of *Plantago lanceolata* mucilage. *Planta Med* 1985; **48**: 293–97.
4. Roklin RE. Mediators of cellular Immunity. In: *Basic and clinical immunology*. Los Altos, CA: Lange Medical Publications 1976: 102–13.
5. Moorehead PS, Nowell PC, Mellman WJ, *et al.* Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res* 1960; **20**: 613–16.
6. Sakagami H, Ikeda M, Unten S, *et al.* Antitumor activity of polysaccharide fraction from pine cone extract of *Pinus parviflore* sieb et Zucc. *Anticancer Res* 1987; **7**: 1153–9.
7. Takeo S, Kado H, Yamamoto H, *et al.* Studies on an antitumor polysaccharide RBS derived from rice bran. II. Preparation and general properties of RON, an active fraction of RBS. *Chem Pharm Bull* 1988; **36**: 3609–13.
8. Bakharova IK, Osokina LI, Revazova ES, *et al.* Effects of plant polysaccharide palyustran on the growth of human tumor transplants in athymic mice. *Vopr Onkol* 1990; **36**: 448–57.
9. Wang DC. Influence of *Astragalus membraneceus* (AM) polysaccharide FB on immunologic function of human periphery blood lymphocytes. *Chung-Hua-Chung-Liu-Tsa-Chih* 1989; **11**: 180–3.
10. Xie SS. Immunoregulatory effect of polysaccharide of *Acanthopanax senticosus* (PAS). I. Immunological mechanism of PAS against cancer. *Chung-Hua-Chung-Liu-Tsa-Chih* 1989; **11**: 338–40.
11. Muller BM, Rosskopf F, Paper DH, *et al.* Polysaccharides from *Nerium oleander*: structure and biological activity. *Pharmazie* 1991; **46**: 657–63.

(Received 10 November 1992; revised version received 10 December 1992; accepted 12 January 1993)