13. Tissue Binding Patterns of Lectins in Premalignant And Malignant Lesions of the Oral Cavity

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Lectins are proteins or glycoproteins mainly of plant origin, the interactions of which can be inhibited or reversed by simple sugars (Pena et al 1981). Lectins have two or more binding sites and can agglutinate cells by binding specific carbohydrate molecule on cell surfaces (Sharon, 1980, Harrison and Chesterton, 1981). Because of the high specificity, the lectins are used for the purifications of proteins and polysaccharides, for lymphoblastogenesis, studies of blood group substances and in membrane studies of normal and cancerous cells (Navogrodsky et al 1975; Miller 1983; Sharon 1984). The difference in carbohydrate structures on cell surfaces were studied earlier by plant lectins from peanut, soyabean and Helix pomatia (Brown and Williams, 1982; Sharon, 1984).

We have earlier reported the isolation and purification of the lectins from the seeds of the Jackfruit and Winged bean (Vijayakumar and Forrester 1986, 1987). Lymphoblastic transformation and mitogenic stimulation of human and rhesus monkey lymphocyte cultures by the extract of jackfruit seeds were reported by Soares et al (1982). The horse-radish peroxidase (HRP) conjugate of the agglutinin from the jackfruit were shown to posses selective staining towards murine and human tissues (Vijayan et al, 1982; Vijayakumar et al, 1985). But the possibility of utilizing the tissue binding property of this lectin as a diagnostic tool for the differential diagnosis of premalignant and malignant lesions has not been explored. Hence the present study was undertaken using tissues of oral leukoplakia, oral submucous fibrosis, verrucous carcinoma and squamous cell carcinoma.

Ten specimens each of histopathologically proved cases of Oral leukoplakia (OL) Oral submucous fibrosis (OSMF), Verrucous carcinoma (VC) and Squamous cell carcinoma (SCC) of the oral cavity were taken up for study. For comparison, ten normal punch biopsy specimens of the oral cavity were also included. Wax embedded tissues were cut into 3-5μ size, dewaxed using 3 changes of xylene and rehydrated using graded alcohol. The JFL and WBL, isolated using a column of immobilized N-acetyl-D-glactosamine as described by Vijayakumar and Forrester (1986), were conjugated to HRP by the method of Nakane and Kawaoi (1974). The purified conjugate was incubated with the rehydrated tissue sections for minutes, washed in three changes of phosphate buffered saline (PBS) and finally incubated in a solution of 30mg% diaminobenzidine dihydrochloride (DAB) in PBS containing 40μl of H_2O_2. The slides were washed in distilled water, counterstained with haemalum, dehydrated with alcohol and mounted. Depending upon the intensity of staining, slides were graded + to +++. The HRP was purchased from Sigma chemical company and DAB purchased from BDH, England.

The physical and chemical properties of the lectins are given in Table-1. In spite of having a common inhibitory sugar, WBL did not stain any of the tissues studied whereas JFL showed varying degrees of staining. The JFL binding patterns of the tissues are given in Table. 2. Trials were carried out using HRP conjugated peanut lectin. The binding pattern was similar to JFL but the intensity was less. Another important observation was that three of the premalignant lesions showed irregular binding pattern similar to that of squamous cell carcinoma.

Walker (1985) demonstrated the selective staining of malignant cells by lectins whereas Howard et al (1981) detected carcinoma associated antigenic alterations in breast cancer by lectin binding. Ree and Kadin (1985) reported that the lectin from Ricinus communis could detect benign histiocytes from malignant histiocytes.

The binding properties of the lectins used in the present study were not investigated earlier in detail. The studies on JFL were mostly on crude preparations (Soares et al, 1982; Vijayan et al 1982), whereas no reports are available on WBL. In this study, the binding shown by JFL towards the premalignant and malignant lesions of the oral cavity were different. The figures clearly demonstrate the binding patterns and is suggestive of their utilization as an immunohistochemical stain.

The results with murine thymus (Rose et al, 1980) and human skin (Vijayakumar et al, 1985) suggest that the lectin from the jackfruit recognizes incomplete nonsialated forms of membrane glycoconjugates. Since these less than fully differentiated forms may be expressed at the surface of malignant cells than their normal counterparts, the JFL may be of use as a diagnostic aid in exfoliative cytology.

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TABLE - 1 PROPERTIES OF JFL AND WBL

	Winged bean lectin	Jack fruit lectins
1. Haemagglutination :	Agglutinates human group A, B and O erythrocytes as well as sheep and rabbit erythrocytes, but not mouse erythrocytes.	Agglutinates human A, B and O erythrocytes as well as sheep, rabbit and mouse erythrocytes.
2. Molecular weight :	A single component of Mol. Wt 35,000, unaffected by reduction	Two iso-lectins of Mol. Wts. 11500 and 15,000, unaffected by reduction.
3. Isoelectric point :	A single band p^I, 4.0	A spread of components between p^I 6.0 and 8.3.
4. Best inhibitor :	N-acetyl-D-galactosamine	N-acetyl-D galoctosamine.
5. Yield of lectin :	6.0mg/gm of defatted meal retaining more than 80% of the haemagglutinating activity	2.0mg/gm of defatted meal retaining more than 75% of the haemagglutinating activity.

6. Amino acid compositions :-

Amino acid	Molar percent WBL	JFL	Amino acid	Molar percent WBL	JFL
Aspartic acid	9.06	9.24	Methionine	0.67	1.16
Threonine	5.57	7.02	Isoleucine	2.35	7.02
Serine	4.40	8.70	Leucine	10.94	6.36
Glutamic acid	13.76	7.08	Tyrosine	3.49	7.43
Proline	10.44	4.51	Phenyl alanine	7.10	6.85
Glycine	2.58	14.73	Histidine	3.05	0.72
Alanine	8.69	2.28	Lysine	10.00	6.41
Valine	6.17	9.13	Arginine	4.03	1.36

TABLE - 2

JFL BINDING PATTERN IN PREMALIGNANT AND MALIGNANT LESIONS OF THE ORAL CAVITY

Tissue	Cytoplasm	Intercellular area	Keratinised area	Cells at basement membrane
Normal	Uniform	Uniform	Nil	Diffuse
(n = 10)	+ (10/10)	++(10/10)		(10/10)
OL	Uniform	Uniform	Uniform	Uniform
(n = 10)	+ (10/10)	+++ (10/10)	++ (6/10)	++(6/10)
			Irregular	Irregular
			+++(4/10)	++(4/10)
OSMF	Uniform	Uniform	Uniform	Uniform
(n = 10)	++(10/10)	+++(10/10)	++(7/10)	++(10/10)
			Irregular	
			++(3/10)	
VC	Focal	Irregular	Irregular	Uniform
(n = 10)	++ (10/10)	+++(10/10)	+++(8/10)	+++(10/10)
			++(2/10)	
SCC	Irregular	Irregular	Irregular	Focal
(n = 10)	+++(10/10)	+++(10/10)	+++(7/10)	+++(10/10)
			++(3/10)	