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European Journal of Pharmaceutics and Biopharmaceutics 53 (2002) 289–292

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

A quantitative evaluation of radiolabelled lectin retention on oral mucosa in vitro and in vivo

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Received 17 July 2001; accepted in revised form 29 January 2002

Abstract

Previous work has identified lectins that bind to the cells present on the oral mucosa for their potential use as a means of retaining a drug delivery system on the mucosal surfaces of the mouth. In this study, a radiolabelling technique was developed to allow the quantification of lectin binding to human buccal cells in vitro, and the retention of the lectins in the oral cavity of a rat model in vivo. Lectins were labelled with ^{99m}Tc using a cyclic diethylene triamine pentaacetic acid conjugation technique. In the in vitro study, human buccal cells were obtained by scraping the inner surface of the cheek. The suspended cells were exposed to the labelled lectin solution for 30 min and after washing with buffer the activity associated with the cells determined. In the in vivo study, male Wistar rats were briefly anaesthetized during which 10 μl of a solution containing labelled lectin was applied into the buccal pouch. At set times the rats were killed and the lower buccal cavity mucosal tissue and tongue dissected out and monitored for bound lectin. The in vitro study indicated that the lectins from *Arachis Hypogaea*, *Canavalia ensiformis* and *Triticum vulgaris* bound to oral mucosal cells. The *T. vulgaris* lectin showed the greatest binding, calculated to be 6.77×10^9 molecules per cell. The in vivo retention of *C. ensiformis* and *T. vulgaris* lectins on rat oral mucosal tissue was also evident. The *T. vulgaris* lectin showed significantly higher levels of retained lectin after 30 min (29.54 ± 4.20 μg SD) on the oral mucosal tissue and 28.37 μg (±2.13 SD) on the tongue and was still detected at similar levels after 2 h. These studies indicate that significant lectin binding to human buccal cells occurs in vitro and retention in an animal model occurs for over 2 h in vivo. The *T. vulgaris* lectin showed most promise for further work.

Keywords: Lectin; Drug delivery; Oral cavity; Radiolabel; *Triticum vulgaris*

1. Introduction

The oral cavity is used as a site for local and systemic drug delivery. Local therapy is used to treat conditions such as gingivitis, oral candidosis, oral lesions, dental caries, and xerostoma. Delivery systems used include mouthwashes, aerosol sprays, chewing gums, bioadhesive tablets, gels and patches [1]. There are two major problems associated with drug therapy within the oral cavity. The first is the rapid elimination of drugs due to the flushing action of saliva [2,3], which may lead to the requirement for frequent dosing. The second is the non-uniform distribution of drugs within saliva on release from a solid or semisolid delivery system [4], which could mean that some areas of the oral cavity might not receive therapeutic levels of drug.

Lectins are ubiquitous natural proteins or glycoproteins that bind reversibly and specifically to carbohydrate moieties of complex glycoconjugates, and were described in a recent review as being 'second generation' bioadhesives [5]. They have been shown to bind, and be retained, on human oral mucosal cells [6,7]. In an in vivo study lectins appeared to persist on oral epithelial surfaces for several hours despite the action of saliva [7,8].

The overall aim of this study was to identify lectins that can be introduced into constructs to target and/or retain diagnostic or therapeutically active agents within the oral cavity for extended periods. In our previous in vitro study we investigated lectin binding to cells from the mucosal surfaces of the human mouth and rat buccal mucosa using subjective and semi-quantitative methods [6]. In this study, a radiolabelling technique was developed to allow the quantification of lectin binding. This was used in this 'proof of concept' study to measure the quantity of lectin binding to cells from the oral epithelial surfaces in vitro, and then the in vivo retention in an animal model. The lectins chosen were those showing promise in previous work [6], namely the n-acetyl-glucosamine/sialic acid specific *Triticum vulgaris* (wheat germ), glucosa/mannose specific *Canavalia ensifor-*

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0939-6411/02/$ - see front matter
PII: S0939-6411(02)00012-7

Table 1
Quantitative study of the in vitro study of ^{99m}Tc-labelled lectins binding to human buccal cells ($n = 3$)

Lectin source	Molecular weight (kDa)	Lectin binding per cell (ng ± SD)	Lectin molecule binding per cell × 10^9 (mean ± SD)
Arachis hypogaea	120	0.36 ± 0.17	1.99 ± 0.88
Canavalia ensiformis	102	0.12 ± 0.02	0.82 ± 0.15
Triticum vulgaris	36	0.40 ± 0.10	6.77 ± 1.81

mis (jack bean) and galactose (and derivatives) specific *Arachis hypogaea* (peanut) lectins.

2. Materials and methods

2.1. Materials

All lectins, stannous chloride dihydride ($SnCl_2$), cyclic diethylenetriamine pentaacetic acid and halothane were obtained from Sigma Chemicals, Poole, Dorset, UK. Male Wistar rats, circa 200 g weight were bred at the University of Portsmouth. NAP-25 columns were obtained from Pharmacia, St. Albans, UK. ^{99m}Tc (as the pertechnetate ion) was obtained as a gift from St. Mary's Hospital, Portsmouth Hospitals NHS Trust, Portsmouth, UK. All other agents were of analytical or high purity quality, and purchased from Sigma.

2.2. Preparation of labelled lectins

^{99m}Tc-labelled lectins were prepared using a technique based on that originally described by Hnatowich et al. [9]. Briefly 10-mg samples of each lectin were mixed with approximately 3.5 mg cyclic diethylene triamine pentaacetic acid (cDTPAA) to give a lectin/cDTPAA ratio of 1:100, and after 5 min agitation rapidly solubilized in 1 ml of acetate buffer, pH 6. After 15 min, 0.1 ml of 3 mg ml^{-1} $SnCl_2$ solution was added and stirred for 15 min. 0.1 ml of a ca. 4.0 MBq ml^{-1} ^{99m}Tc solution was then added, stirred for 15 min and the radiolabelled lectin isolated using a NAP-25 fast desalting column. Two activity peaks were eluted from the column. The second did not show absorption at 280.1 nm indicating the absence of protein, and was therefore associated with the unbound ^{99m}Tc. The first fraction eluted was then passed through a second desalting column to confirm only one activity peak, containing lectin and the DTPA conjugate, was now present. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis, using the procedure of Laemmli [10] was also completed to ensure that the molecular weight of the conjugated lectin remained the same as that of the unconjugated lectin and significant degradation had not occurred. The lectin concentration was found by measuring its absorbance at 280.1 nm and comparing with a standard calibration curve; from this, the activity per standard weight of lectin was calculated from the sample count, correcting for radioactive decay.

For the in vivo study the lectin was concentrated (from 2 to 0.5 ml) by high-speed centrifugation at $5000 \times g$ for 30 min.

2.3. In vitro human buccal cell study

Human buccal cells, obtained by scraping the inner surface of the cheek of a healthy male volunteer as described by Nantwi et al. [6], were suspended in 10 ml 0.05 M Tris-buffered saline (pH 7.6) containing 1 mM calcium chloride (TBS). A 1-ml sample was taken, stained with trypan blue and the cell concentration found using a haemocytometer. The remaining 9 ml of the cell suspension was centrifuged at 1500 rpm for 15 min, the supernatant was discarded, and the cells washed by resuspending in TBS and centrifuging on three further occasions. The cells were then suspended in 0.5 ml of the labelled lectin solution, left for 30 min, then centrifuged and washed with TBS on four further occasions. The activity associated with the cell suspension was determined using a well counter and Scalar Ratemeter (SR 7) (Nuclear Enterprises Ltd). From the known activity associated with each milligram of lectin, and the number of cells present in the suspension determined by haemocytometry, the activity associated with each cell was calculated.

2.4. In vivo rat study

All animal experimentation was completed after obtaining the appropriate United Kingdom government licences. Rats were lightly anaesthetized using halothane for an approximately 30-s period, during which time 10 μl of a

Table 2
Binding of ^{99m}Tc labelled *Canavalia ensiformis* lectin to the lower oral cavity tissue and tongue of the rat expressed in μg, and the percentage bound, 10, 30 and 60 min after application ($n = 3$)

Tissue source	10 min after application		30 min after application		60 min after application	
	Weight (μg ± SD)	Percent ± SD	Weight (μg ± SD)	Percent ± SD	Weight (μg ± SD)	Percent ± SD
Lower oral tissue	7.80 ± 2.66	3.33 ± 1.14	5.39 ± 0.45	2.31 ± 0.18	4.78 ± 0.58	2.04 ± 0.24
Tongue	15.67 ± 7.79	6.70 ± 3.33	5.03 ± 0.63	2.15 ± 0.27	5.39 ± 1.55	2.30 ± 0.66

Table 3
Binding of ^{99m}Tc-labelled *Triticum vulgaris* lectin to the lower oral cavity tissue and tongue of the rat expressed in μg, and the percentage bound, 30, 60 and 120 min after application ($n = 3$)

Tissue source	30 min after application		60 min after application		120 min after application	
	Weight (μg) ± SD	Percent ± SD	Weight (μg) ± SD	Percent ± SD	Weight (μg) ± SD	Percent ± SD
Lower oral tissue	29.54 ± 4.20	13.57 ± 1.94	28.08 ± 0.87	12.91 ± 0.40	32.86 ± 3.87	15.11 ± 1.78
Tongue	28.37 ± 2.13	13.04 ± 0.98	34.19 ± 4.26	15.69 ± 1.96	31.37 ± 4.35	14.41 ± 2.00

solution containing 15–25 mg ml^{-1} labelled *C. ensiformis* or *T. vulgaris* lectin was applied to the oral buccal pouch (between the lower incisors, gingiva and buccal mucosa). The rats were kept for set time intervals, during which they were allowed free access to food and water, then killed, and the lower buccal cavity mucosal tissue, tongue and (in one case only) the stomach tested for the presence of associated lectin without any further processing. All experiments were completed in triplicate and the data statistically analysed using 'Minitab 13' software (Minitab Inc., State College, PA, USA).

3. Results and discussion

All three lectins bound to the isolated human buccal cell surfaces. 1.99×10^9 molecules of the lectin from *A. hypogaea*, 0.82×10^9 molecules of the lectin *C. ensiformis* and 6.77×10^9 molecules of the lectin from *T. vulgaris* were calculated to bind to each buccal cell (Table 1). The quantity of lectin binding was significantly different ($P < 0.05$, one-way analysis of variance), with the *T. vulgaris* lectin showing significantly higher binding than the other two ($P < 0.05$, Tukey's multiple comparison test). In our previous work using microdensitometry [6], the *T. vulgaris* lectin also gave the greatest apparent binding to buccal cells. This work [6] also suggested that binding is due to a specific lectin-like interaction. A very tentative extrapolation of this level of binding to a buccal cell in vitro, would suggest *T. vulgaris* and *A. hypogaea* lectin binding to a 100-cm^2 oral mucosal surface in the mg range.

The in vivo retention study using the rat as an animal model was employed to identify if two lectins that were found to bind to rat and human mucosal cells in vitro [6] will also be retained for extended periods in vivo. A decrease in *C. ensiformis* lectin binding was seen between 10 and 30 min (Table 2) but binding was seen to persist for 60 min with no further significant decrease (one-way analysis of variance, Tukey's multiple comparison test). It might be that the quantity retained after 30 min represents truly bound lectin, unbound lectin being washed out prior to this. It is interesting to note that the in vivo retention reflects the in vitro binding of these lectins, suggesting that binding is by a lectin-like interaction. As expected, the clearance of the lectin was by swallowing and entrance into the stomach, where circa 25% of the *C. ensiformis* lectin was recovered 10 min after administration.

Significantly higher levels ($P < 0.05$, Tukey's multiple comparison test) of the *T. vulgaris* lectin were evident after 30 and 60 min, and were also present 2 h after application at similar levels (Table 3) ($P > 0.05$, one-way analysis of variance). It is worth noting that this study measured binding to tissues readily accessible to dissection only, which would constitute less than half of the total area of the oral cavity. It is likely therefore that much higher levels of *T. vulgaris* lectin were retained overall in the oral cavity. No significant reduction in binding was evident after 2 h, and it is probable that retention for several hours more would occur, and this should be further investigated.

This study is the first quantitative assessment of lectin binding within the oral cavity and indicates that retention for at least a 2 h period is achievable in vivo, confirming the work of Gibbons and Dankers [7] in humans. These lectins have promise in providing the means by which therapeutic or diagnostic constructs may be retained for extended periods within the oral cavity. In further work, confirmation that in vivo binding is by a lectin-like interaction will be sought, and then the formulation and evaluation of lectin-carrier constructs considered.

Acknowledgements

We would like to thank the Medical Physics Department, St. Mary's Hospital, Portsmouth Hospitals NHS Trust, Portsmouth, UK for the kind gift of the ^{99m}Tc solutions.

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