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Short communication

Studies on the uptake of tomato lectin nanoparticles in everted gut sacs☆

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Abstract

Tomato lectin (TL) is a bioadhesive glycoprotein that has been shown to bind selectively to the small intestine epithelium. When bound to polystyrene microspheres, intestinal uptake occurs not only through the gut associated lymphoid tissue (GALT) but also through normal enterocytes. In this study, the everted gut sac model was used to compare the rates and quantities of intestinal uptake of tomato lectin and that of TL coupled to microspheres. Using bovine serum albumin (BSA) and BSA coupled to microspheres as comparators. Uptake is time and concentration dependent. Transfer of TL from the lumen to the serosa was 3.9 ng/mg per h whereas that of BSA was 0.5 ng/mg per h. Hence uptake of tomato lectin was 7-fold higher than BSA. The rate of uptake of TL coupled microspheres was 41.5 ng/mg per h, which was 4-fold higher than microspheres coupled to BSA (11.8 ng/mg per h). The uptake of TL conjugated microspheres was shown to be inhibited by *N*-acetyl-D-glucosamine tetramer $[GlcNac]_4$.

Keywords: Tomato lectin; Oral drug delivery; Everted gut sacs

The future of oral drug delivery, particularly of peptides, will depend on the increasing efficiency and specificity of delivery of drugs by suitable delivery systems. Among possible strategies, microparticles represent versatile carrier systems able to improve the pharmacokinetic profile of numerous peptides while conferring protection against the hostile and degrading milieu of the intestine. Increased specificity of interaction between carrier

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particles and absorptive epithelia make this process more efficient.

Tomato lectin (TL) is a bioadhesive glycoprotein that has been shown to bind selectively to the small intestinal epithelium (Naisbett and Woodley, 1994). It has been found that when bound to polystyrene microspheres, intestinal uptake occurred not only through the gut associated lymphoid tissue (GALT) but also through normal enterocytes (Hussain et al. 1997). After 5 days continuous dosing, the extent of absorption was 15 fold higher via the enterocytes than by way of the Peyer's patches. These 'engineered' particles were able to gain entry into the circulation via villous tissue and as this represents a much higher targetable surface area than lymphoid tissue, it is of interest. The TL interaction with mucus has been reported in vitro (Irache et al., 1996).

In this communication, the intestinal uptake of TL itself and that of TL conjugated microspheres is measured. Uptake was compared to that of bovine serum albumin (BSA) and BSA conjugated microspheres. In all cases, uptake was found to be time and concentration dependent. Transfer of TL from the lumen to the serosa was 3.9 ng/mg per h whereas that of BSA was 0.5 ng/mg per h. TL conjugated microspheres uptake rate was 41.5 ng/mg per h, which was 4-fold higher than the uptake of BSA coupled microspheres (11.8 ng/mg per h).

Tomato lectin (Vector Laboratories, UK) was radiolabelled using the Bolton-Hunter technique (Bolton and Hunter, 1973) and was coupled to 500 nm fluorescent carboxylated nanoparticles (Polysciences®, Warrington, USA) using the carbodiimide method (Hussain et al. 1997), obtaining a yield of 3–4 μg of TL/mg of microspheres. SDS-PAGE electrophoresis and erythrocyte agglutination tests indicated lectin stability.

Controls included BSA coupled particles, BSA and TL. To study the specificity of enterocyte binding, a TL common inhibitor consisting of the tetramer of *N*-acetyl-D-glucosamine $[GlcNAc]_4$, was employed. The ^{125}I-labelled ligands were employed for quantification.

Everted gut sac preparation and incubation was performed as described by Naisbett and Woodley (1994). The extent of uptake of BSA coupled nanospheres (20 μg/ml) as well as BSA and TL (2 μg/ml) within 2 h was studied. To establish the specificity of uptake, an inhibitor of TL, *N*-acetyl-D-glucosamine tetramer $[GlcNac]_4$, was incubated

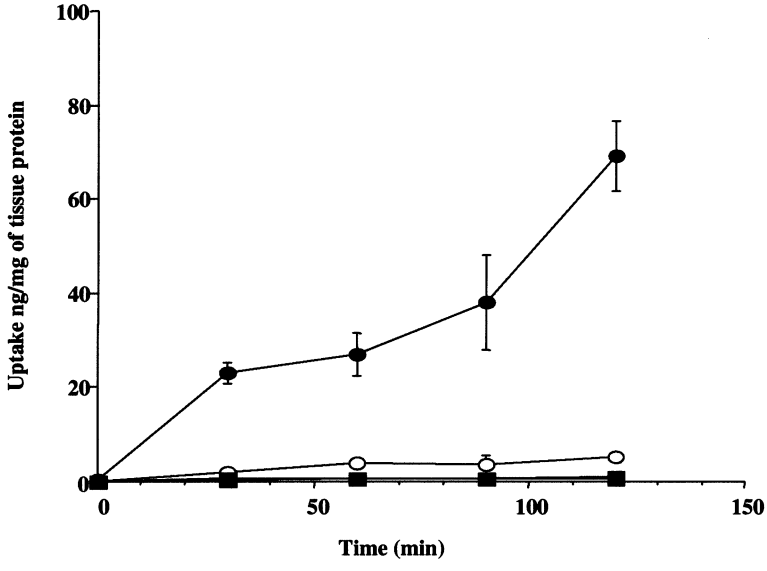

Fig. 1. Serosal and tissue accumulation of tomato lectin (TL) and bovine serum albumin (BSA) in rat everted gut sacs. –●–, TL tissue uptake; –○–, TL serosal transfer, –■–, BSA tissue uptake, –□–, BSA serosal uptake. Serosal uptake and tissue accumulation increase with time ($n = 9$).

Table 1
Rates of uptake of the different substrates tested expressed in different units

Substrate	Concentration[a] (μg/ml)	Rate of uptake[b] (ng/mg of protein per h) ± S.E.M.		Endocytic index (μl/mg of protein per h) ± S.E.M.		% Uptake/h	
		Serosa	Tissue	Serosa	Tissue	Serosa	Tissue
Controls							
Tomato lectin	2.0	3.91 ± 0.9	31.31 ± 3.1	1.50 ± .3	13.43 ± 4.0	0.003	0.039
	5.0	10.01 ± 3.3	99.67 ± 10.9	2.09 ± .7	19.93 ± 2.7	0.006	0.060
	10.0	35.11 ± 6.6	200.31 ± 27.0	3.50 ± 0.7	20.03 ± 2.2	0.011	0.060
	20.0	30.00 ± 3.2	195.12 ± 9.8	1.50 ± 0.2	9.75 ± .5	0.005	0.029
	30.0	29.00 ± 10.7	200.32 ± 30.6	0.97 ± 0.4	6.67 ± 1.9	0.003	0.020
BSA	2.0	0.50 ± 0.1	0.72 ± .3	0.25 ± 0.0	0.35 ± .0	0.001	0.001
	5.0	0.45 ± 0.0	2.35 ± .5	0.09 ± 0.0	0.42 ± 0.0	0.000	0.001
	10.0	1.61 ± 1.0	6.00 ± .7	0.16 ± 0.1	0.31 ± .0	0.000	0.002
	20.0	4.03 ± 0.6	8.02 ± 2.0	0.20 ± 0.0	0.40 ± .0	0.001	0.001
	30.0	6.107 ± 2.3	9.41 ± .9	0.20 ± 0.1	0.30 ± 0.0	0.001	0.001
Microspheres							
TL-microspheres	5.0	4.215 ± 0.5	291.721 ± 77.0	0.840 ± .1	58.340 ± 15.4	0.003	0.175
	10.0	12.856 ± 2.8	387.196 ± 25.1	1.200 ± .3	38.710 ± 2.5	0.004	0.116
	20.0	22.72 ± .9	1680.15 ± 137.2	0.680 ± 0.2	84.005 ± 6.9	0.003	0.252
	50.0	28.21 ± 1.9	3684.45 ± 745.0	0.560 ± 0.0	73.688 ± 14.9	0.002	0.221
BSA-microspheres	5.0	3.612 ± 1.0	58.52 ± 7.8	0.720 ± .2	11.700 ± 7.5	0.002	0.035
	10.0	3.80 ± 0.4	159.35 ± 17.6	0.380 ± 0.0	15.930 ± 9.1	0.001	0.048
	20.0	11.81 ± 3.1	529.61 ± 43.7	0.393 ± 0.1	17.650 ± 0.0	0.001	0.053
	50.0	14.51 ± 0.9	653.4 ± 173.1	0.290 ± 0.0	13.068 ± 3.8	0.001	0.039

[a] In the case of the controls (tomato lectin, TL and bovine serum albumin, BSA) concentration relates to the amount of protein used, whereas in the case of microsphere it refers to the concentration of microspheres used and not that of ligand.

[b] Rate of uptake in the case of controls represents the uptake of the proteins whereas in the case of microspheres it represents the uptake of microspheres.

with TL- and BSA-coupled microspheres through time at 10 × excess in molar concentration.

Uptake has been expressed in terms of an Endocytic Index, defined as the volume of culture medium (μl or μg of substrate) whose contained substrate is captured per mg cell protein/h. The endocytic index normalises for the effects of variation in specific radioactivity or specific enzymatic activity and for the amount of tissue present (Pratten et al., 1980).

The binding and uptake of the two control molecules, ^{125}I-labelled TL and ^{125}I-BSA is shown in Fig. 1. From this Figure and from Table 1 it can be seen that tissue uptake in both cases increases with linearly with time at rates of 31.3 ng/mg of tissue protein per h and 0.5 ng/mg of tissue protein per h, respectively. TL accumulation into the tissue is thus shown to be some 52-fold that of BSA. Transfer to the serosal fluid was much slower in the case of TL (3.9 ng/mg of tissue protein per h) and similar in the case of BSA (0.5 ng/mg of tissue protein per h). TL uptake into the serosal fluid was 8-fold higher than that of BSA.

The uptake of TL conjugated microspheres and BSA conjugated microspheres, into the tissue and into the serosal fluid is represented in Fig. 2. It can be seen that serosal uptake for both systems increased linearly with time, the same being true for tissue uptake except that after 60 min incubation, saturation occurs. The calculated rates of uptake are listed in Table 1. When comparing serosal uptake BSA-coupled microspheres slower than TL-coupled microspheres by a factor of 2,

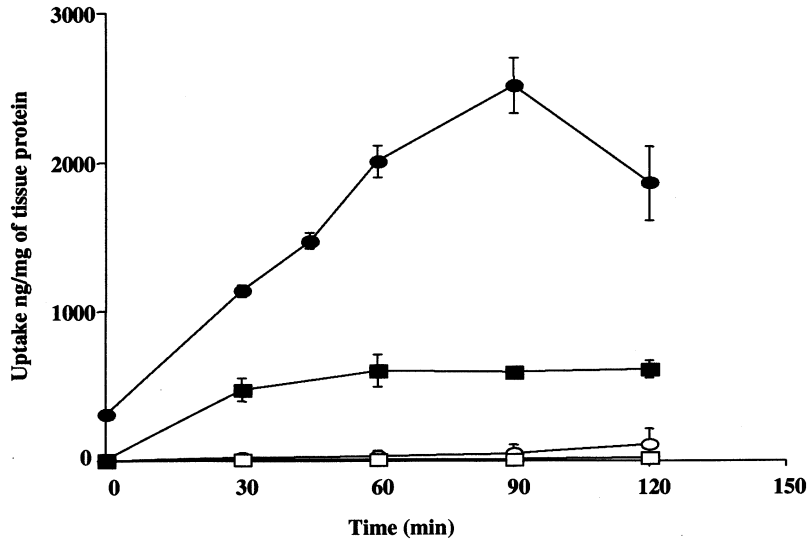

Fig. 2. Serosal and tissue uptake of tomato lectin (TL) and bovine serum albumin (BSA) conjugated microspheres (20 μg/ml). –■–, represents BSA conjugated microspheres accumulation in the tissue; –□–, represents BSA conjugated to microspheres serosal uptake; –●–, is TL conjugated microspheres tissue accumulation; and –○–, is serosal transfer of TL conjugated microspheres. TL conjugated microspheres tissue and serosal uptake increases linearly with time, whereas BSA conjugated microspheres display saturation of tissue accumulation after 30 min ($n = 9$).

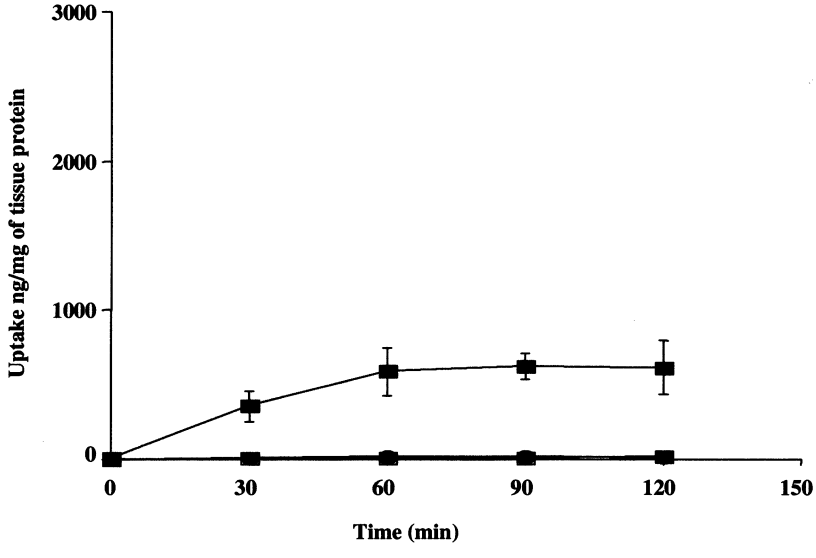

Fig. 3. Serosal and tissue uptake of tomato lectin (TL) and bovine serum albumin (BSA) conjugated microspheres (20 μg/ml) in the presence of × 10 molar excess of *N*-acetyl-D-glucosamine tetramer $[GlcNAc]_4$. –■–, represents BSA-conjugated microspheres accumulation in the tissue; –□–, BSA conjugated to microspheres serosal uptake; –●–, is TL conjugated microspheres tissue accumulation; and –○–, serosal transfer of TL conjugated microspheres. TL conjugated microsphere tissue and serosal uptake are inhibited by 95 and 64%, respectively, in the presence of the competing sugar. In contrast, BSA conjugated microspheres display no inhibition either in serosal nor in tissue uptake ($n = 9$).

while tissue uptake of BSA-coupled microspheres was a factor of 3 slower.

Results obtained when the conjugates were incubated with $[GlcNAc]_4$ (Fig. 3), show that uptake was reduced only in the case of TL conjugated microspheres, where tissue uptake was 8.2 ± 2.1 ng/mg of tissue protein per h (representing a 95% inhibition) and serosal uptake was 14.9 ± 2.8 ng/mg of tissue protein per h (a 64% inhibition). These data confirm the specific nature of lectin binding to the mucosa. $[GlcNAc]_4$ had no noticeable effect on the uptake of BSA conjugated microspheres.

In terms of rates and amount of uptake quantified in this study, microspheres are more readily taken up than the proteins alone. Only ~ 0.34 μg of TL or BSA is present in microspheres at a concentration of 20 μg/ml. If 20 μg/ml of TL conjugated microspheres is taken as an example, an uptake of 22.72 ± 4.90 ng/mg per h is found in the serosa and 1680.15 ± 137.20 ng/mg/h in the tissue. In contrast, in the case of Tomato lectin at 20 μg/ml we found 30.01 ± 3.2 and 195.12 ± 9.8 ng/mg per h in the serosa and tissue, respectively. This comparison is useful in terms of drug delivery since it highlights the increased uptake of proteins such as TL when coupled to nanospheres. There are two factors to consider: the conformation that TL acquires once bound to the nanosphere (since once TL is on the surface of these systems it may display more binding sites than when it is by itself adopting a tertiary conformation) and the effect of nanospheres in mediating uptake.

Naisbett and Woodley (1995) have cast some doubt on the use of prolonged doses of TL for oral drug delivery since it elicits an immunological response (serum IgG and intestinal IgA) after only a oral dose of 2.5 μg of TL/mice. In this paper it was observed that very small amounts of lectin once coupled to microspheres are required to obtain enhanced uptake. High doses of TL coupled to microspheres could be used as immunoprophylactic agents and also as carriers for vaccine-relevant antigens such as cholera toxin, staphylococcal enterotoxin B and others.

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