

Lectin-mediated transport of nanoparticles across Caco-2 and OK cells

G.J. Russell-Jones ^{a,*}, H. Veitch ^a, L. Arthur ^b

^a *Biotech Australia Pty Ltd., PO Box 20, Roseville, NSW, 2069, Australia*

^b *Westmead Hospital, Sydney, Australia*

Received 30 November 1998; received in revised form 16 July 1999; accepted 19 July 1999

Abstract

Recent experiments by a number of workers have suggested that it may be possible to use various targeting molecules, which bind to the intestinal epithelium, to promote the uptake and transport of nanoparticles from the intestine to the circulation. We have used commercial nanoparticles to examine the effect of size, density and inhibitors on uptake of lectin-coated nanoparticles by epithelial cells. The degree of uptake was most influenced by the density of lectin on the particle, with size and type of lectin being less important. Uptake could be inhibited by the presence of specific sugars or free lectin. These studies should provide a good basis for the design of targetable biodegradable drug-loadable particles suitable for oral delivery. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Lectin; Caco-2; OK; Nanoparticles; Oral drug delivery

1. Introduction

Oral administration of peptides and proteins, while being arguably the most desirable route of administration of these pharmaceuticals, suffers from the disadvantage that these molecules are rapidly degraded by proteolytic enzymes in the gut. Furthermore, only a small quantity (less than 1 percent) of molecules that survive degradation are able to cross the epithelial cell layer and enter the circulation. In an attempt to protect peptides

and proteins from proteolysis many workers have tried to incorporate these molecules into nano- or microparticles. They have then tried to use the non-specific antigen-sampling activity of the intestinal M cells to promote uptake of these drugladen particles from the intestine (O'Hagan, 1990; Smith et al., 1995; Lomotan et al., 1997). While this strategy is reported to protect these molecules from proteolysis, the quantity of material taken up from the intestine is still low, resulting in less than one percent of the administered dose entering the circulation. There are several reasons for the low level of uptake of the particles. Firstly, the majority of intestinal M cells are located at the base of villi, and as such are located in a restricted site of the intestinal wall. Secondly,

* Corresponding author. Tel.: + 61-2-9928-8834; fax: + 61-2-9928-8899.

E-mail address: greg_russell_jones@mail.bioaust.com.au (G.J. Russell-Jones)

as the work of Desai et al. (1996) and Frey et al. (1996) suggests, the intestinal epithelial cell glyco-calyx acts to limit access of larger (400 nm to 1 μ m) nanoparticles to the M cells. Thirdly, apart from the M cells, which are present in only low numbers (less than one percent of all enterocytes), most enterocytes do not take up particles non-specifically.

Recent studies by Russell-Jones and others (De Aizpurua and Russell-Jones, 1987; Pusztai, 1989; Russell-Jones, 1996; Russell-Jones et al., 1997; Gabor and Wirth, 1997) have suggested that molecules such as lectins, pili, haemagglutinins, toxins and invasins, which bind to intestinal epithelial cells, can be used as targeting molecules to initiate binding to these cells. This in turn delays the transit of antigens and proteins through the gut. In some cases these molecules can even initiate uptake of the targeting molecules, as well as proteins linked to them, into and across the intestinal epithelial cells (De Aizpurua and Russell-Jones, 1987; Russell-Jones, 1996; Russell-Jones et al., 1997; Gabor and Wirth, 1997). In an extension to these findings, it has also been demonstrated that it may be possible to coat the surface of nanoparticles, with the above targeting agents, and thereby cause these molecules to bind to intestinal epithelial cells. In some cases, such binding may be sufficient to stimulate the enterocytes to endocytose the 'coated nanoparticles' and transport the particles across the cell and into the circulation (Russell-Jones and Westwood, 1992; Naisbett et al., 1993; Florence et al., 1995; Irache et al., 1996; Gabor and Wirth, 1997; Hussain et al., 1997; Russell-Jones et al., 1997, 1992, 1998).

To better understand the requirements for lectin-mediated uptake of nanoparticles by epithelial cells, this study was performed using an in vitro cell culture model of intestinal epithelial cells. Various parameters were examined including; variability in uptake with different lectins, the effect of lectin density, inhibition with soluble inhibitors and the effect of size on uptake. Three different molecules with different binding specificities were used in the study. Wheat germ agglutinin (WGA), a dimeric protein composed of two identical subunits that contain identical binding sites for N-acetylglucosamine (NAcGlu), ConA,

which binds to α -D-mannose and LTB, the binding subunit of *E. coli* heat labile toxin, LT, which binds to GM-1 ganglioside and galactose.

2. Materials and methods

2.1. Materials

ConA, WGA, GM-1 ganglioside, α -D-mannose and NAcGlu were from Sigma. LTB was purified from the culture supernatant of *E. coli* expressing recombinant LTB using methods described previously (Russell-Jones and de Aizpurua, 1987).

2.2. Cell culture studies

Lectin-mediated uptake and transcellular transport were studied in two cell lines, the intestinally derived human colon carcinoma cell line, Caco-2, and the opossum kidney carcinoma cell line (OK cell line) (Ramanujam et al., 1991; Russell-Jones, 1996). Both of these cell-lines exhibit polarized transport when cultured on semi-permeable membranes in two-chambered tissue culture wells. The intestinal cell line differs from the kidney cell line in that it is more heterogeneous and is microvillated, whereas the kidney line is more homogeneous, does not possess micovili and shows little variation from passage to passage.

The Caco-2 and OK cell lines were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g l⁻¹ glucose, 25 IU penicillin and streptomycin, 1 mM L-glutamine, and 10% foetal bovine serum (DME + FCS) at 37°C in an atmosphere of 5% CO₂. For transport experiments cells were grown in T75 flasks until 80–100% confluent. Following washing with PBS, adherent cells were removed by treatment with 10 μ g ml⁻¹ trypsin 10 mM EDTA for 5 min. Cells were immediately diluted 1.5-fold into fresh culture medium and used to seed cultures at 3.4×10^5 cells/well in 12 well/plate Costar transwells. Cells were grown for further 12–24 days prior to testing. Costar transwells had a membrane insert size of 12 mm and a pore size of 3 μ M, with an apical chamber volume of 0.4 ml

and a basal chamber volume of 1 ml. Cells generally reached confluence within 10 days. Transepithelial resistance (TEER) measurements were made of monolayers and only cultures with a TEER greater than 250 ohms cm^{-2} were used for experimentation.

2.3. Preparation of FITC-labeled proteins

LTB, WGA and bovine serum albumin (BSA) were modified with fluorescein isothiocyanate (FITC) by reacting a FITC solution (40 mg ml^{-1} in EtOH) with a 10 mg ml^{-1} solution of protein in 0.1 M carbonate buffer pH 9.5, at a ratio of 1:25 FITC: protein (w/w). The reaction was allowed to proceed for 2 h at room temperature, before removal of unreacted FITC by extensive dialysis against distilled water.

2.4. Conjugation of lectins to Polysciences nanoparticles

Fluorescent YG nanoparticles (50, 100, 200 and 500 nm in size) were obtained from Polysciences. These nanoparticles contain a fluorescein-like fluorophore and have surface carboxylic acids groups suitable for chemical modification. Particles were activated by the addition of EDAC/NHS for 10 min after which LTB, ConA or WGA (dissolved in fresh 2% bicarbonate) was added to the particles and allowed to react for 2 h. Activated carboxyl groups on the particles were blocked with glycine overnight. The particles were then centrifuged, washed three times with 0.1 M pH 9.5 carbonate, resuspended in distilled water and dialysed extensively against distilled water. Following dialysis the nanoparticles were sonicated extensively to generate a uniform suspension of individual particles.

2.5. Preparation of particles modified with various lectin densities

Particles were prepared with increasing surface densities of LTB, WGA or ConA, by incubating EDAC/NHS-activated nanoparticles (12.5 mg ml^{-1}) with lectins at 2.5, 5, 10 and 20 mg ml^{-1} . Unbound lectins were removed by precipitation in

0.1 M carbonate buffer pH 9.5, followed by three washes in the precipitating buffer. Finally the nanoparticles were resuspended by sonication into distilled water and extensive dialysis against distilled water.

2.6. Transport studies

Prior to transport studies, the apical culture medium was removed from transwell cultures and the apical surface of the Caco-2 and OK cells washed once with buffer (PBS). The basolateral wells were then re-fed with 1 ml of complete medium. Dilutions of FITC-labeled lectin solutions and fluorescent Polysciences nanoparticles, +/– lectin coating (0.25 ml in DMEM + FCS) were added to the chamber of triplicate wells and the cultures incubated at 37°C overnight. Apical and basal media were then removed from the wells and the cells washed with PBS (3X), followed by one wash with 5 mM EDTA, pH 5.0 for 30 min. The apical and basal chambers were assayed for the presence of nanoparticles by measurement of the entrapped fluorochrome as follows. Bound and internalized fluorescence was measured in the lysate of cells incubated with a solution of 2% SDS, 50 mM EDTA pH 8.0 for 30 min at room temperature. The membrane inserts were excised from the transwell and the fluorescence associated with the filter assessed by lysis of the nanoparticles with 50% acetone to release the entrapped fluorochrome from the nanoparticles. Particles that had been transported to the basal chamber were assayed by measurement of basal media. All samples were adjusted to pH 8.0 with 1 M Tris–HCl pH 9.0 prior to fluorescence measurement. Fluorescence was measured on a Bio-Tek Instruments Microplate Fluorescence Reader (FL500) at $\text{Ex}_{485 \text{ nm}}$ and $\text{Em}_{530 \text{ nm}}$. Data is expressed as the percentage of the total fluorescence added to the wells, which was found in each fraction. For all measurements care was taken to ensure that fluorescence was measured on a constant volume of sample placed in a square bottom ELISA plate (Nunc). A linear relationship was observed between fluorescence intensity and nanoparticle concentration over the ranges tested (data not shown). In separate studies using radio-

labelled ligands, we have also found a direct correlation between the quantity of surface bound ligand and the amount of measurable fluorescence (data not shown).

2.7. Uptake and transport of FITC-labeled lectins by *Caco-2* cells

Caco-2 cells were incubated with 200 µg FITC-labeled LTB (LTB-FITC), 200 µg FITC-labeled LTB pre-incubated with 2 mg of GM₁-ganglioside (LTB – FITC + GM₁), 200 µg FITC-labeled LTB plus 2 mg LTB (LTB – FITC + LTB), or 200 µg FITC-labeled BSA (BSA – FITC). In separate experiments, *Caco-2* cells were incubated with 200 µg FITC-labeled WGA (WGA-FITC), 200 µg FITC-labeled WGA pre-incubated with 2 mg of NAcetylGlucosamine (WGA-FITC + NAcGlu), 200 µg FITC-labeled WGA plus 2 mg WGA (WGA – FITC + WGA), 200 µg FITC-labeled WGA plus 2 mg BSA (WGA – FITC + BSA), or 200 µg FITC-labeled BSA (BSA – FITC). After 18 h, the cells were treated as described in the 'Transport studies' section of the Materials and methods. Data is expressed as the percentage of FITC which could be found within the cells (Cells) or within the filter inserts (Inserts), or as the total of Cells + Inserts.

2.8. Effect of exogenous free lectin on the uptake and transport of WGA, LTB or ConA-coated 50 nm Polysciences particles by *Caco-2* and OK cells

Caco-2 and OK cells were incubated with 500 µg plain or WGA. LTB or ConA coated 50 nm Polysciences YG nanoparticles. Particles were pre-incubated with 2mg of cold lectin for 20 min prior to addition to cell cultures. After 18 h, the cells were treated as described previously.

3. Results

3.1. Uptake and transport of FITC-labelled WGA and LTB

Previous studies have revealed that LTB is able

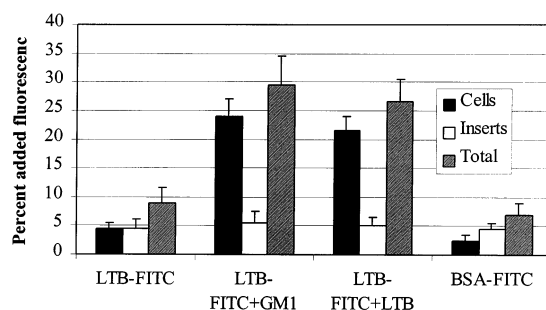


Fig. 1. Uptake and transport of FITC-labeled LTB by *Caco-2* cells. The cells were treated as described in the Materials and Methods. Data is expressed as the percentage of FITC which could be found within the cells (Cells) or within the filter inserts (Inserts), or as the total of Cells + Inserts.

to bind to, and be internalized by, the intestinal epithelial cells of mice, as well as *Caco-2* cell monolayers (Lazorova et al., 1993). A comparison of uptake and transport of WGA and LTB modified with FITC revealed that levels of LTB-FITC uptake were very similar to those of the control, FITC-BSA (BSA, 5%, Fig. 1). In contrast, FITC-WGA (30% of the added dose) was found to be endocytosed by the cell, although little fluorescence was transported to the cell and into the basal chamber (Fig. 2).

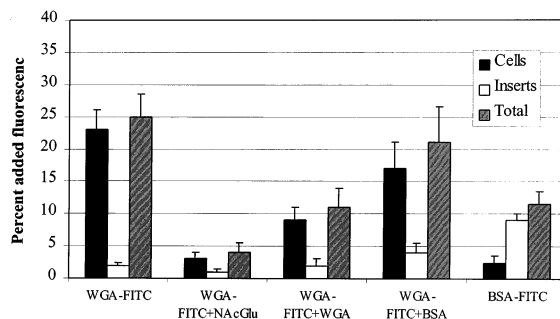


Fig. 2. Uptake and transport of FITC-labeled WGA by *Caco-2* cells. The cells were treated as described in the Materials and Methods. Data is expressed as the percentage of FITC which could be found within the cells (Cells) or within the filter inserts (Inserts), or as the total of Cells + Inserts.

3.2. Inhibition of uptake of FITC-modified lectins by excess unlabeled lectin

The uptake of FITC–WGA was found to be specific, as the addition of excess WGA significantly reduced the uptake of WGA–FITC (from 30 to 8%, Fig. 2). Uptake was also reduced by the presence of NAcGlu, but was not affected by the addition of BSA. In contrast to the results with FITC–WGA, the uptake of LTB–FITC was enhanced in the presence of excess LTB (from 4.8 to 7.8%, Fig. 1), or by the addition of excess GM1-ganglioside. These somewhat paradoxical results can possibly be explained by a low density of the GM-1 ganglioside on Caco-2 cells. Thus, addition of extra LTB or the GM-1 ganglioside may increase the frequency of interaction of LTB with the cell, thereby increasing the rate of endocytosis of the FITC-labeled LTB. These findings suggest that there is some requirement for multipoint binding of the LTB to the surface of the cell in order to elicit the formation of endocytic pits and subsequent internalisation of the LTB. Similar enhancement of uptake of an endogenous protein, CT (cholera toxin) was found by Moss and others (Moss et al., 1976; Joseph et al., 1979), who demonstrated greater killing of target cells by CT upon addition of increasing levels of GM-1 ganglioside. The differences in uptake of FITC–WGA and FITC–LTB may be a result of the different types of ligands to which each binds. Thus, it is known that LTB binds to the glycolipid, GM-1 ganglioside, while WGA binds to NAcGlu-modified proteins.

3.3. Uptake and transport of lectin-modified nanoparticles

Having found that lectins could bind to and be internalized by Caco-2 cells the possibility that lectin-coated nanoparticles could be endocytosed by these cells was investigated. Levels of uptake and transport of nanoparticles modified with lectins were generally equal to or greater than those seen with FITC–lectins, which is perhaps surprising considering the large size of the lectin-modified nanoparticles (Fig. 3).

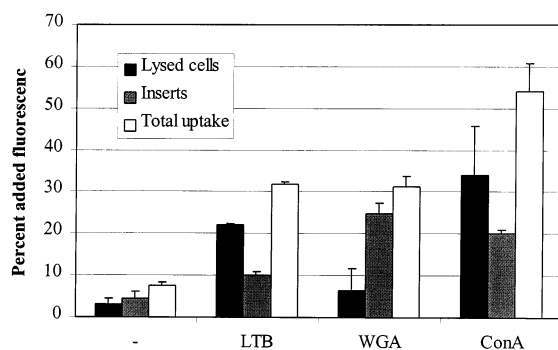


Fig. 3. Uptake and transport of WGA, LTB or ConA-coated 50 µg Polysciences particles by Caco-2 cells. Caco-2 cells were incubated with 500 µg plain or WGA, LTB or ConA-coated 50 nm Polysciences YG nanoparticles. After 18 h, the cells were treated as described in the Materials and Methods. Data is expressed as the percentage of added particles (as determined from fluorescence) which could be found within the cells (Cells) or within the filter inserts (Inserts), or as the total of Cells + Inserts.

Greatest uptake was observed with ConA-coated nanoparticles with many of the particles being transported across the cells and into the filter inserts. Lower levels of uptake were observed with WGA and LTB-coated nanoparticles, with both particles being transported through the cells and into the insert. The difference in the level between the three molecules may reflect a slight difference in the degree of modification of the particles by these molecules (see later). With both the LTB and WGA coated nanoparticles, there was greater transport of the particles to the insert, than the transport observed with the FITC–lectins. It is therefore possible that the intracellular trafficking route undertaken by the particles differs from that of the soluble lectin. The difference in trafficking may be due either to particles binding to different ligands than the soluble lectin, or possibly due to enhanced receptor clustering that would be expected with the high level of multi-point binding expected from the high density of lectin coating the nanoparticles.

3.4. Effect of size on uptake and transport of lectin-modified nanoparticles

Similar levels of uptake of WGA-coated nanoparticles were observed for particles ranging

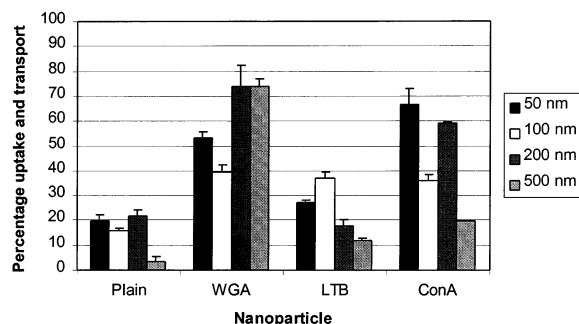


Fig. 4. Effect of size on the uptake and transport of WGA, LTB or ConA coated Polysciences particles by Caco-2 cells. Caco-2 cells were incubated with 500 μ g plain or WGA, LTB or ConA-coated Polysciences YG nanoparticles of 50, 100, 200 or 500 nm in size. After 18 h, the cells were treated as described in the Materials and Methods. Data is expressed as the percentage of added particles (as determined from fluorescence) which could be found both within the cells and within the filter inserts.

size from 50 to 500 nm. In contrast the level of uptake of LTB or ConA coated particles decreased as particle size increased from 50 to 500 nm (Figs. 4 and 5). The level of uptake of WGA, LTB or ConA modified nanoparticles was similar for both Caco-2 and OK cells (cf. Figs. 4 and 5). High levels of uptake were seen for particles ranging in size from 50 to 500 nm. It is not known why the level of uptake of the 200 and 500 nm WGA particles (70%) was higher than for the

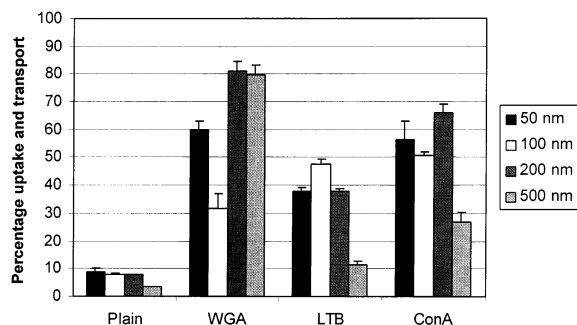


Fig. 5. Effect of size on the uptake and transport of WGA, LTB or ConA-coated Polysciences particles by OK cells. OK cells were incubated with 500 μ g plain or WGA, LTB or ConA-coated Polysciences YG nanoparticles of 50, 100, 200 or 500 nm in size as described in Figure 4.

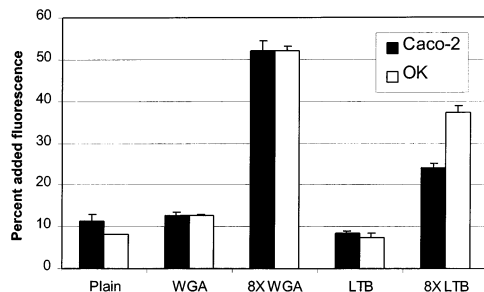


Fig. 6. Effect of lectin substitution on the uptake and transport of WGA and LTB-coated 50 nm Polysciences particles by Caco-2 and OK cells. Caco-2 and OK cells were incubated with 500 μ g plain or WGA or LTB-coated 50 nm Polysciences YG nanoparticles. Particles had been prepared with the lectins at either 2.5 mg ml^{-1} (1X) or 20 mg ml^{-1} (8X). After 18 h, the cells were treated as described in the Materials and Methods. Data is expressed as the percentage of added particles (as determined from fluorescence) which could be found both within the cells and within the filter inserts.

smaller 50 and 100 nm particles (~ 40 –50%), although it is possible that there were slight differences in the amount of lectin bound to the various particle preparations. In this regard all particles were checked for binding to appropriately glycosylated agarose beads, however, no observable difference in binding was noted (results not shown).

3.5. Effect of lectin density on surface of particles on uptake

Uptake of WGA, LTB or ConA-modified nanoparticles was critically dependent upon the quantity of lectin used in the preparation of the lectin-modified nanoparticles, presumably reflecting the degree of substitution of the nanoparticles by these molecules. Thus, particles prepared using lectins at 2.5 mg ml^{-1} (1X) were able to elicit uptake that was only marginally higher than unmodified nanoparticles. Increasing the quantity of lectin used for the preparation of the nanoparticles (20 mg ml^{-1} ; 8X) resulted in a linear increase in uptake to levels 5 and 4 times that of unmodified particles for WGA and LTB, respectively (Fig. 6 data shown for 1 and 8X concentrations).

3.6. Inhibition of uptake of lectin-modified particles by soluble inhibitors

The specific nature of the uptake of the lectin-modified nanoparticles was demonstrated by the finding that co-administration of soluble lectin was able to inhibit the uptake of WGA and ConA modified particles (Fig. 7). The uptake of WGA and ConA modified nanoparticles was also inhibited by excess NAcGlu or D-mannose, respectively. In contrast, the uptake of LTB-modified nanoparticles was slightly enhanced by the addition of GM₁-ganglioside Fig. 8.

4. Discussion

For many years immunological dogma has stated that uptake of protein antigens (and indeed microparticles) from the gut occurs through non-specific antigen sampling of the gut by the 'M' cells present on the dome epithelium of the Peyer's Patches. This has biased the thought of many workers and has led them to concentrate on using this route (the 'M' cell) for the oral delivery of proteins and microparticles to the circulation (Hillery et al., 1995a,b; Jani et al., 1995; Smith et

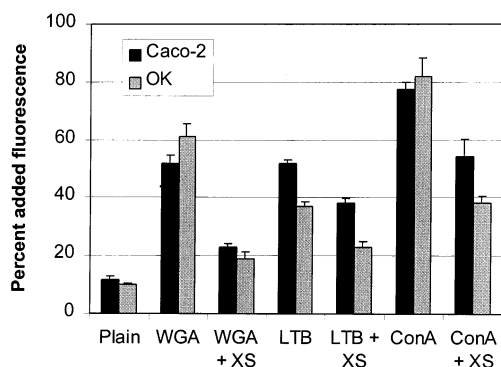


Fig. 7. Effect of exogenous free lectin on the uptake and transport of WGA, LTB or ConA-coated 50 nm Polysciences particles by Caco-2 and OK cells. Cells were treated with WGA-, LTB-, or ConA-coated nanoparticles either alone or in the presence of 2 mg of unconjugated lectin (+XS) as described in the Materials and Methods. Data is expressed as the percentage of added particles (as determined from fluorescence) which could be found within the Cells + Inserts.

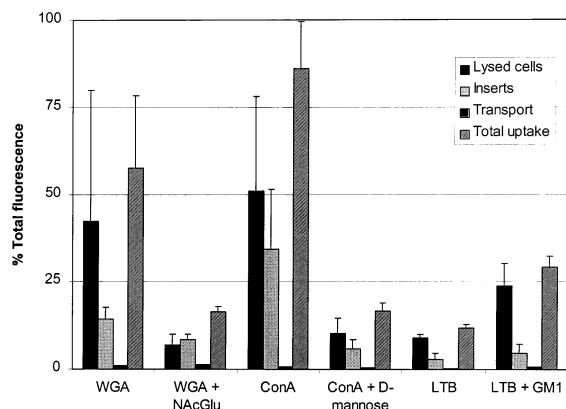


Fig. 8. Effect of specific sugars on the uptake and transport of WGA, ConA or LTB-coated 50 nm Polysciences particles by Caco-2 and OK cells. Caco-2 and OK cells were incubated with 500 µg plain or WGA, ConA or LTB-coated 50 nm Polysciences YG nanoparticles. Particles were pre-incubated with 8 mg of NAcGlu or D-Mannose, or 2 mg GM1 ganglioside before addition to cell cultures. After 18 h, the cells were treated as described in the Materials and Methods. Data is expressed as the percentage of added particles (as determined from fluorescence) which could be found within the Cells (Lysed cells), the Inserts (Inserts), the basal chamber (Transport), or total uptake (lysed + inserts)(Total uptake).

al., 1995), and even to develop targeting systems for the 'M' cells (Jepson et al., 1996). It has become apparent, however, through the studies of Russell-Jones et al. and other workers, that many proteins are absorbed from the gut through normal intestinal epithelial cells that are separate from the dome epithelium (De Aizpurua and Russell-Jones, 1987; Pusztai, 1989; Lazorova et al., 1993; Lindner et al., 1994; Russell-Jones, 1994, 1996, 1997; Haltner and Lehr, 1997; McClean et al., 1998). A common property of the proteins that use this alternate uptake pathway is that they are able to bind to the intestinal epithelial cells and initiate uptake and subsequent transcytosis of these molecules and of molecules covalently linked to them (Russell-Jones, 1994, 1996; Russell-Jones et al., 1997; Russell-Jones, 1998; Russell-Jones and de Aizpurua, 1987). The binding properties of these molecules (lectins, toxins, pili, haemagglutinins, invasins, etc.) has been used to delay the intestinal transit time of both proteins and microparticles (Caston et al., 1990; Lehr et al., 1992; Ponchel et al., 1994a,b; Pusztai, 1995; Irache et al., 1996).

In an extension to the above findings several experimenters have shown that it may be possible to use the binding activity of lectins, invasins and other similar molecules to stimulate the uptake and transport of nanoparticles (Russell-Jones and Westwood, 1992; Naisbett et al., 1993, 1994; Jeyanthi et al., 1995; Pusztai, 1995; Hussain et al., 1997; Russell-Jones, 1997, 1998; Lemoine and Preat, 1998; Easson et al., 1997).

In the studies reported here, it has been shown that it is possible to stimulate the *in vitro* uptake of nanoparticles using three different lectins, LTB, WGA and ConA, each of different binding specificities. The extent of uptake and transcytosis of the lectin coated nanoparticles was greatly influenced by the quantity of lectin bound to the surface of the particles, with highest densities being preferred. It is interesting to note that a similar correlation has been observed between the density of surface binding molecules and the degree of virulence of many types of pathogenic bacteria (Russell-Jones and Gotschlich, 1984). The extent of uptake of the various nanoparticles was similar for the sizes 50, 100 and 200 nm, while a reduction in uptake was noted for LTB and ConA coated 500 nm particles. This could possibly reflect a limitation in the extent of coating of the nanoparticles by these lectins, or a possible size restriction in the endocytic vesicle responsible for nanoparticle uptake. Uptake of nanoparticles was found to be highly specific and was readily inhibited by co-administration of free lectin, or of the specific sugar or ganglioside to which the lectin was known to bind.

Although there are few examples of oral delivery of pharmaceuticals using toxin binding subunits, or lectins, it has been shown that oral administration of these molecules elicits high titres of serum antibody to both the lectins/toxins and to haptens and proteins covalently linked to them (De Aizpurua and Russell-Jones, 1987; Russell-Jones et al., 1992; Lindner et al., 1994; Russell-Jones, 1994, 1996, 1997, 1998), suggesting that these molecules could also be used as vehicles for oral drug delivery. The repeated usefulness of these molecules for oral drug delivery, however, is questionable, as all molecules studied so far are highly immunogenic and hence would elicit an

antibody response to the carrier. These antibodies could either block the binding of the lectins/toxins, or alternatively the antibodies raised could render some individuals highly allergic to the immunizing food lectins and hence elicit severe systemic anaphylaxis as a result of ingesting even small quantities of the lectins in food. The use of these lectins as a delivery system would thus be contraindicated in persons with known food allergies. It is possible, however, that the immunogenicity of these targeting molecules could be greatly reduced, either by genetically altering or removing the T-cell epitopes, or alternatively by identifying smaller, less immunogenic fragments of the lectins that maintain their binding activities.

5. Conclusions

The oral delivery of peptides and proteins within lectin-coated nanoparticles appears to be an ideal method for protecting these molecules against digestion as well as increasing the uptake of the pharmaceutical through the targeting action of the lectins. These studies have demonstrated that there may be a range of targeting molecules, including LTB, ConA and WGA, which are suitable for delivery of nanoparticles. These molecules are able to elicit targeting and uptake of nanoparticles in a range of sizes from 50 to 500 nm. The level of targeting and uptake is directly proportional to the amount of targeting agent attached to the particles. Targeting was also found to be a specific phenomenon as it was greatly reduced in the presence of excess free lectin, or specific sugar. The challenge for the future will be to manufacture drug-loaded nanoparticles, with sufficient surface density of lectins for uptake, and to be able to subsequently control the rate of release of the drug from the nanoparticle, once it has crossed the cell and entered the circulation.

References

- Caston, A.J., Davis, S.S., Williams, P., 1990. The potential of fibrial proteins for delaying intestinal transit of Oral drug

- delivery systems. Proc. Int. Symp. Control. Rel. Bioact. Mater. 17, 313–314.
- De Aizpurua, H.J., Russell-Jones, G.J., 1987. Oral vaccination: Identification of classes of proteins which provoke an immune response upon oral feeding. J. Exp. Med. 167, 440–451.
- Desai, M.P., Labhasetwar, V., Amidon, G.L., Levy, R.J., 1996. Gastrointestinal uptake of biodegradable microparticles: Effect of particle size. Pharm. Res. 13, 1838–1845.
- Easson, J.H., Jahn, D., Lehr, C.-M., 1997. Utilizing bacterial mechanisms of cell entry for the design of bioinvasive drug carriers. Proc. Int. Symp. Control. Rel. Bioact. Mater. 24, 101–102.
- Florence, A.T., Hillery, A.M., Hussain, N., Jani, P.U., 1995. Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. J. Control. Rel. 36, 39–46.
- Frey, A., Giannasca, K.T., Weltzin, R., Giannasca, P.J., Reggio, H., Lencer, W.I., Neutra, M.R., 1996. Role of glycolyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: Implications for microbial attachment and oral vaccine targeting. J. Exp. Med. 184, 1045–1059.
- Gabor, F., Wirth, M., 1997. Lectin-mediated bioadhesion: wheat germ agglutinin and Solanum tuberosum lectin as targeting moieties in particulate drug delivery systems. Europ. J. Cell Biol., 46 74, 12.
- Haltner, E., Lehr, C.-M., 1997. Two distinctive classes of plant lectins and their utilization for controlled drug delivery to epithelial cells. Proc. Int. Symp. Control. Rel. Bioact. Mater. 24, 49–50.
- Hillery, A.M., Toth, I., Florence, A.T., 1995a. A novel oral nanoparticulate delivery system, I: Development and in vitro studies. Proc. Int. Symp. Control. Rel. Bioact. Mater. 22, 266–267.
- Hillery, A.M., Toth, I., Florence, A.T., 1995b. A novel oral nanoparticulate delivery system for the oral absorption of peptides, II: In vivo studies. Proc. Int. Symp. Control. Rel. Bioact. Mater. 22, 268–269.
- Hussain, N., Jani, P.U., Florence, A.T., 1997. Enhanced uptake of tomato lectin-conjugated nanoparticles in the rat. Pharm. Res. 14, 613–618.
- Irache, J.M., Durrer, C., Duchene, D., Ponchel, G., 1996. Bioadhesion of lectin-latex conjugates to rat intestinal mucosa. Pharm. Res. 13, 1716–1719.
- Jani, P.U., Yamashita, F., Nomura, T., Takakura, Y., Florence, A.T., Hashida, M., 1995. Biliary excretion of polystyrene microspheres after oral and parenteral administration to rats. Proc. Int. Symp. Control. Rel. Bioact. Mater. 22, 198–199.
- Jepson, M.A., Clark, M.A., Foster, N., Mason, C.M., Bennett, M.K., Simmons, N.L., Hirst, B.H. 1996. Targeting to intestinal M cells. J. Anat. 507–516.
- Jeyanthi, R., Kodak, J., Van Hamont, J., Cassels, F., McQueen, C.E., Reid, R.J., 1995. Preliminary assessment of a microencapsulated polyvalent oral vaccine against enterotoxigenic *E. coli*. Proc. Int. Symp. Control. Rel. Bioact. Mater. 22, 276–277.
- Joseph, K.C., Stieber, A., Gonatas, N.K., 1979. Endocytosis of cholera toxin in GERL-like structures of murine neuroblastoma cells pretreated with GM ganglioside. J. Cell Biol. 81, 543–554.
- Lazorova, L., Sjolander, A., Russell-Jones, G.J., Lindner, J., Artusson, P., 1993. Intestinal Tissue Distribution and Epithelial Transport of the Oral Immunogen LTB. the B Subunit of *E. coli* Heat-labile Enterotoxin. J. Drug Targeting 1, 331–340.
- Lehr, D.M., Bouwstra, J.A., Kok, W., Noach, A.B.J., de Boer, G., Junginger, H.E., 1992. Bioadhesion by means of specific binding of tomato lectin. Pharm. Res. 9, 547.
- Lemoine, D., Preat, V., 1998. Polymeric nanoparticles as delivery system for influenza virus glycoproteins. J. Control. Rel. 54, 15–27.
- Lindner, J., Geczy, A.F., Russell-Jones, G.J., 1994. Identification of the Site of Uptake of the *E. coli* Heat-labile Enterotoxin, LTB. Scand. J. Immunol 40, 564–572.
- Lomotan, E.A., Brown, K.A., Speaker, T.J., Offita, P.A., 1997. Aqueousbased microcapsules are detected primarily in gut-associated dendritic cells after oral inoculation of mice. Vaccine 15, 1959–1962.
- McClellan, S., Prosser, E., Meehan, E., O'Malley, D., Clarke, N., Ramtoola, Z., Brayden, D., 1998. Binding and uptake of biodegradable poly-DL-lactide micro- and nanoparticles in intestinal epithelial. Eur. J. Pharm. Sci., 6, 153–163.
- Moss, J., Fishman, P.H., Manganiello, V.C., Vaughan, M., Brady, R.O., 1976. Functional incorporation of ganglioside into intact cells: Induction of cholera response. Proc. Nat. Acad. Sci. 73, 1034–1037.
- Naisbett, B., Wagenaar, B., Bouwstra, J.A., Junginger, H.E., 1993. Lectins as adjuvants in oral microsphere vaccine delivery systems. Proc. Intern. Symp. Control. Rel. Bioact. Mater. 20, 337–338.
- Naisbett, B., Wagenaar, B., Bouwstra, J.A., Junginger, H.E., 1994. A lectin adjuvant for oral vaccine delivery. Proc. Int. Symp. Control. Rel. Bioact. Mater. 21, 885–886.
- O'Hagan, D.T., 1990. Intestinal translocation of particulates-implications for drug and antigen delivery. Adv. Drug Deliv. Rev. 5, 265–285.
- Ponchel, G., Irache, J.M., Durrer, C., Duchene, D., 1994a. Covalent coupling of lectins to colloidal particles. Proc. Int. Symp. Control. Rel. Bioact. Mater. 21, 312.
- Ponchel, G., Irache, J.M., Durrer, C., Duchene, D., 1994b. Lectin-latex conjugates for specific bioadhesion. Proc. Int. Symp. Control. Rel. Bioact. Mater. 21, 800–801.
- Pusztai, A., 1989. Transport of proteins through the membranes of the adult gastrointestinal tract-a potential for drug delivery? Adv. Drug Deliv. Rev. 3, 215–228.
- Pusztai, A., 1995. Lectin-targeting of microparticles to different parts of the gastrointestinal tract. Proc. Int. Symp. Control. Rel. Bioact. Mater. 22, 161–162.
- Ramanujam, K.S., Seetharam, S., Ramasamy, M., Seetharam, B., 1991. Expression of cobalamin transport proteins and cobalamin transcytosis by colon adenocarcinoma cells. Am. J. Physiol. 260, 6416–6422.
- Russell-Jones, G.J., Gotschlich, E.C., 1984. Identification of

- protein antigens of Group B streptococci, with special reference to the Ibc antigens. *J. Exp. Med.* 160, 1476–1484.
- Russell-Jones, G.J., de Aizpurua, H.J., 1987. Mucosal immunogens. In: Mestecky, J., McGhee, J.R., Bienenstock, J., Ogra, P.L. (Eds.), *Recent Advances in Mucosal Immunology*, 216B. Plenum, New York.
- Russell-Jones, G.J., Westwood, S.W., 1992. Oral delivery systems for microparticles. (USPA 07/956.003: WO 92/17167; PCT/AU92/00141) (Granted)
- Russell-Jones, G.J., Lindner, J., de Aizpurua, H., 1992. The use of lectins for oral delivery. *Proc. Int. Symp. Control Rel. Bioact. Mater.* 19, 40–41.
- Russell-Jones, G.J., Oral vaccination with lectins and lectin-like molecules. In: D.T. O'Hagan (Ed.), *Novel Delivery Systems for Oral Vaccines*, Boca Raton, CRC Press, 1994, 225–240.
- Russell-Jones, G.J., 1996. The potential use of receptor-mediated endocytosis for oral drug delivery. *Adv. Drug Deliv. Rev.* 20, 83–97.
- Russell-Jones, G.J., Walker H. Killinger, S., Starling, S., 1998. Lectin-mediated transport of nanoparticles. *Proc. International Symposium on Controlled Release of Bioactive Materials*.
- Russell-Jones, G.J., Lindner, J., Killinger, S., Arthur, L., 1997. Use of recombinant LTB and other lectins as carriers for oral vaccination with haptens, viruses and nanoparticles. *Proc. 9th. Int. Congr. Mucosal Immunology*.
- Russell-Jones, G.J., 1998, Carrier-Mediated Transport for Oral Drug Delivery. In: Edith Mathiowitz (Ed.), *Encyclopedia of Controlled Drug Delivery*, Wiley, New York (submitted for publication)
- Smith, M.W., Thomas, N.W., Jenkins, P.G., Miller, N.G.A., Cremaschi, D., Porta, C., 1995. Selective transport of microparticles across Peyer's Patch follicle-associated M cells from mice and rats. *Exp. Physiol.* 80, 735–743.