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Minireview

Particle uptake and translocation across epithelial membranes

N. W. THOMAS[2], P. G. JENKINS[3], K. A. HOWARD[1], M. W. SMITH[4], E. C. LAVELLE[1], J. HOLLAND[1] AND S. S. DAVIS[1]

[1] *Department of Pharmaceutical Sciences, University of Nottingham,* [2] *Department of Human Morphology, University Medical School, Nottingham,* [3] *Proteus Molecular Design Ltd, Macclesfield and* [4] *Department of Physiology, Royal Free Hospital School of Medicine, London, UK*

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ABSTRACT

Oral delivery of drugs and vaccines has many advantages over other routes of administration. For example, for vaccination, enteric delivery may result in the induction of a mucosal immune response against pathogens which colonise and invade the mucosa. However, the oral delivery of peptide or protein drugs or antigens is beset with problems, such as gastrointestinal breakdown of labile molecules, low level of macromolecular absorption and, for vaccines, the poor immune response usually elicited by orally administered soluble antigens. Investigations are therefore in progress to develop means of increasing intestinal absorption and decreasing digestion of orally administered molecules. Molecules can be incorporated into biodegradable microparticles to reduce the effect of gut secretions and to enable the absorption of bioactive agents in an unaltered form. The uptake of microparticulates through the gut wall is accepted as a true biological phenomenon but the mechanism and route of uptake have not been established. Furthermore, in general, only small numbers of microparticles are translocated across epithelial membranes, possibly making these systems inappropriate for drug or vaccine delivery. This paper reviews particle uptake across the gastrointestinal tract and describes studies carried out to determine whether a humoral response can be elicited following oral administration of an antigen associated with biodegradable poly(DL lactide-coglycolide) microparticles. The use of lipid delivery vehicles to enhance microparticle uptake and the selective transport of microspheres across M cells is also described.

Key words: Gastrointestinal tract; drug and vaccine delivery.

INTRODUCTION

The vertebrate gastrointestinal tract represents an important interface between nutrients, other extraneous substances and the interior milieu of the animal. In addition to its role in nutrient digestion and absorption, the digestive tract occupies a vital defence position, responding to incessant immunological challenge via a number of specific and nonspecific processes (Walker, 1982). The function of the epithelium is highly complex being influenced by endocrine, paracrine, stromal and immune elements (McKay & Perdue, 1993).

Many studies have demonstrated that macromolecules can be absorbed through the gastrointestinal tract in sufficient quantities to produce a local immune response, contradicting previous suggestions that macromolecules are completely reduced to their component monomers by gut secretions prior to their uptake (Owen & Ermak, 1990). On this evidence, the oral delivery of drugs and vaccines can be regarded as a potential means for achieving therapeutic and prophylactic effects for a number of conditions. For vaccination, enteric delivery may result in the induction of a mucosal immune response against pathogens which colonise and invade the mucosa. However, the oral delivery of peptide/protein drugs or antigens is beset with problems, such as gastrointestinal breakdown of labile molecules, low levels of macromolecular absorption and, for vaccines, the poor immune response usually elicited by orally administered soluble antigens. Consequently, numer-

Correspondence to Dr J. Holland, Department of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, UK.

ous investigations are underway to develop strategies to increase intestinal absorption and to reduce digestion of orally delivered molecules.

To reduce the impact of gut secretions and to ensure the absorption of bioactive agents in an unaltered form, molecules may be incorporated into biodegradable microparticles. The success of oral delivery of microparticles relies on the capacity of the gastrointestinal tract to absorb microparticulate materials. Although the uptake of microparticulates through the gut is accepted as a true biological phenomenon, the mechanism of uptake is debatable and no accepted route of particle uptake has been confirmed. Many groups believe that uptake is a function carried out by membranous/microfold (M) cells in the Peyer's patches of the mammalian gut. However, other groups claim that uptake across the villous epithelium occurs, via damaged regions or by a paracellular route (Kreuter, 1991; O'Hagan, 1994). Furthermore controversy exists about quantifying the level of uptake across the gut, with different investigators reporting widely variable results.

UPTAKE OF PARTICLES ACROSS THE GASTROINTESTINAL TRACT

In previous investigations, we have provided evidence of particle uptake across the gastrointestinal tract (Howard et al. 1994). Using both rats and rabbits, fluorescent or gold labelled polystyrene microparticles were introduced into closed intestinal loops which incorporated a visible Peyer's patch. Fluorescence and transmission electron microscopy were used to investigate the distribution of particles in Peyer's patches and submucosal tissue of the rabbit and rat. Microscopic analysis of the patch after exposure to particles for 20–40 min showed that the location of particles after uptake was dependent on size, with larger particles (0.94 μm) remaining at the dome epithelium whilst the smaller particles (0.1 μm) were found along the serosal surface. Studies were also carried out to assess the extent and rapidity of microparticle uptake across the rat intestinal epithelium. By cannulating the superior mesenteric lymph duct and using a sensitive flow cytometric assay, the uptake of fluorescent microparticles across Peyer's patches was quantified (Jenkins et al. 1994). Although the number of particles absorbed was very small, uptake into the Peyer's patch and delivery into lymph was rapid, within 5 min of administration. Detection of particles within the mesenteric lymph indicated that particles of 0.94 μm in size delivered to the proximal small intestine were absorbed to a greater extent than either 0.11 μm diameter particles delivered proximally or, interestingly the same 0.94 μm particles delivered distally in the small intestine. These results agree with studies which indicated a correlation between the size and extent of absorption of microparticles (reviewed by Lavelle et al. 1995). The number of particles detected, however, was in sharp contrast to the findings of Jani et al. (1990, 1992) who described uptake of 34% of 50 nm microparticles in rats after oral administration for 10 d, and Alpar et al. (1989) who reported that 39% of 1.1 μm particles were found in the bloodstream of the rat, 45 min after oral administration.

It needs to be pointed out that the term 'uptake' of particles for gut tissues may include quantification both of adsorbed and absorbed particles. Therefore the high figure reported by Jani et al. (1990, 1992) for particle uptake, is perhaps an over-estimate of the levels of actual absorption through the gut.

HUMORAL IMMUNE RESPONSE TO ORALLY ADMINISTERED MICROENCAPSULATED ANTIGEN

The uptake of a small number of microparticles is likely to be inappropriate as a delivery mechanism for a therapeutic dose of a drug, but it might be adequate as a mechanism for stimulating a significant immune response to an orally delivered, microencapsulated antigen. In an in vivo study to investigate the humoral immune response to orally administered equine influenza virus, association of the virus with poly(DL lactide-coglycolide) microparticles (1 μm and 30 μm) did not greatly enhance the immune response following oral delivery of the complexes to mice. Although this finding was disappointing, the poor response does not invalidate the general strategy of oral administration as factors such as the breakdown in the gastrointestinal tract of virus associated with the surface of the microparticle, may have influenced our results. Indeed, oral immunisation with antigen incorporated in microparticles has been demonstrated to induce systemic and secretory antibody responses (Eldridge et al. 1989; Challacombe et al. 1992). Clearly the formulation of the microparticle-antigen complex plays a crucial role in the outcome of this strategy and research in this area is ongoing.

THE USE OF LIPID DELIVERY VEHICLES TO ENHANCE MICROPARTICLE UPTAKE

There is considerable interest in strategies to enhance the interaction of microparticles with M cells of Peyer's patches and their uptake across the gut. These

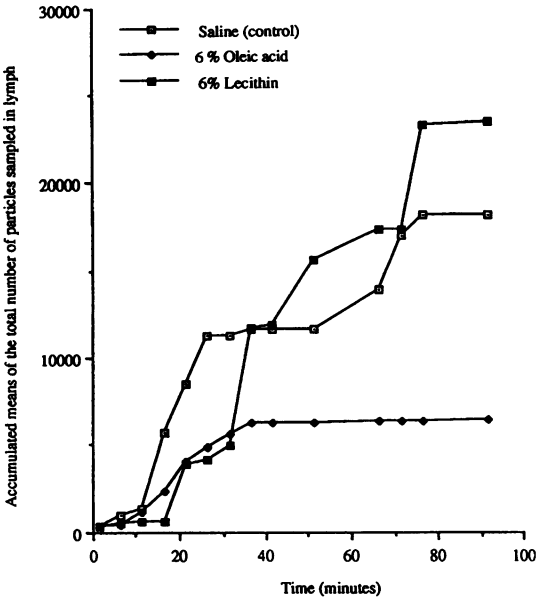

Fig. 1. Accumulated means of the total number of polystyrene microparticles detected in lymph at various time points following their delivery into rat intestinal gut loops with either saline 6% oleic acid or 6% lecithin.

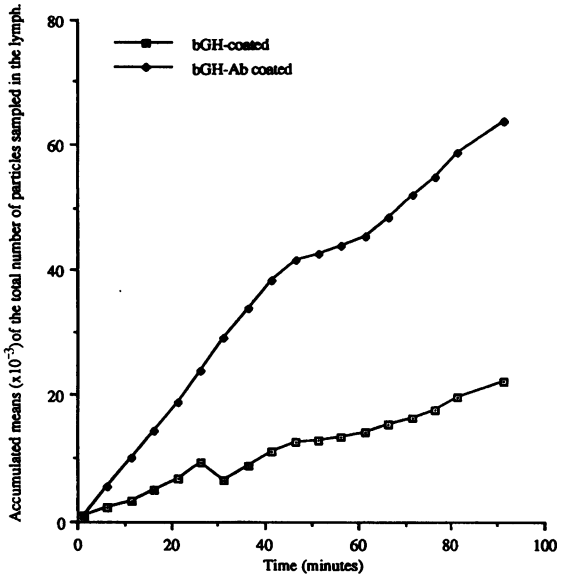

Fig. 2. Accumulated means of the total number of bGH-coated and bGH-Ab coated polystyrene microparticles detected in lymph at various time points following their delivery into rat intestinal gut loops (Smith et al. 1995).

approaches have included the use of lectins, antibodies and the administration of microparticles in milk (Pappo et al. 1991, Hussain et al. 1994; LeRay et al. 1994). We set out to establish if mixing of polystyrene microparticles (0.5 μm) with lipid delivery vehicles could enhance their translocation into the mesenteric lymph of rats. Using conventional representation of uptake data, we have always found difficulty in interpreting the dynamics of particle uptake (Jenkins et al. 1994). However, by resorting to a plot of cumulative means which is in keeping with all 'area under the curve' analysis for comparison, more consistent effects became apparent (Fig. 1). Our findings show that there are significant differences between the numbers of polystyrene microparticles detected in the lymph of rats following their delivery in saline, 6% oleic acid and 6% lecithin. When delivered with lecithin, uptake of particles was significantly greater than in the saline controls, whereas with oleic acid, uptake of particles was significantly less. It is not clear why the 2 lipid vehicles had such a profound and different effect on the uptake of microparticles but the findings may reflect the specific interactions of lipids with membranes.

SELECTIVE TRANSPORT OF MICROSPHERES

A study was undertaken to examine how coating polystyrene micropheres (0.5 μm) with different proteins affected their ability to be taken up by M cells (Smith et al. 1995). Binding and uptake of fluorescent polystyrene microspheres by M cells was determined using confocal microscopy. Flow cytometry was then used to test for the selectivity of microsphere appearance in mesenteric lymph. An interesting feature of this study was that in rats, both surface binding and the uptake of microspheres coated with bovine growth hormone-antibovine growth hormone antibody (bGH-Ab) to M cells was greater than that observed with binding of microspheres coated with bovine growth hormone (bGH) alone. The data from confocal microscopy was in agreement with the results of similar studies in mice. The difference in particle uptake was also apparent in the data from lymph samples from rats (Fig. 2). These finding demonstrate the potential ability of antibodies to increase the uptake of particles across the small intestine.

CONCLUSIONS

Our studies indicate that the absorption of microparticles across the Peyer's patches of rats, mice and rabbits can occur rapidly as microparticles can be detected in the lymph soon after intestinal delivery. Results from the study of Smith et al. (1995), highlight the importance of the M cell surface as the main discriminator for selective transport. However, since the mechanism and accepted route of particle uptake is not fully understood, research in this area must continue.

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