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Short communication

Distribution of a lipidic 2.5 nm diameter dendrimer carrier after oral administration

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Abstract

The biodistribution of a lipidic peptide dendrimer has been studied after oral administration to female Sprague–Dawley rats (180 g, 9 weeks old). Uptake by gut epithelial tissue of the radiolabelled dendrimer molecule (mol. wt. 6300; diameter 2.5 nm; log $P = 1.24$) was studied in rats after a single oral dose by gavage (14 mg/kg). The maximum levels of dendrimer observed were 3% (blood), 1.5% (liver), 0.1% (spleen), 0.5% (kidneys), 15% (small intestine) and 5% (large intestine). Approximately 6% of a single administered dose (28 mg/kg) was recovered from the entire gastrointestinal tract while 1% was absorbed via the small intestine lymphoid tissue after 3 h; after 12 h, 0.1% was detected. The maximum uptake by the non-lymphoid small intestine was 4% of the dose after 3 h. After 12 h, 0.3 and 4% dendrimer was measured in the lymphoid large intestine and the non-lymphoid large intestine, respectively. The total percentage of the administered dose absorbed through the lymphoid tissue was comparatively greater than through the non-lymphoid tissue of the small intestine with respect to organ weight after 3 and 24 h.

Keywords: Dendrimers; Lipoamino acid; Lipidic peptide dendrimer; Colloidal carriers; Lymphoid intestine; Non-lymphoid intestine; Peyer's patches

1. Introduction

The mucosal surfaces represent the major portal of entry of infectious agents, i.e. bacteria and

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viruses, into the body and the site at which many organisms exert their pathophysiological effects. The small intestine contains lymphoid aggregates, Peyer's patches, imbedded along its antimesenteric aspect, the locus in neonates of antigen uptake. It has been suggested that as colloidal carriers can be absorbed by way of the Peyer's patches, this is a route to enhance the oral absorption of encapsulated bioactive agents to minimise enzymatic degradation in the lumen or the brush border region and/or in the cell. Carriers should have the ability to protect labile drugs from degradation, reduce non-specific interactions with food proteins and allow enhanced absorption across the intestinal epithelium. The proposed mechanisms of particulate uptake include persorption (Volkheimer, 1968, 1975), endocytosis by enterocytes (Bockman and Winborn, 1966), paracellular transport (Aprahamian et al., 1987), uptake by intestinal macrophages (Wells et al., 1988) and uptake through the gut associated lymphoid tissue (GALT) (LeFevre et al., 1978a,b, 1980; Jani et al., 1989; LeFevre et al., 1989; Eldridge et al., 1990; Jani et al., 1990, 1992a,b; Florence and Jani, 1993; Hillery et al., 1994). There has been, to date, some differences in estimates of uptake across the gut. Although relatively high levels of uptake have been achieved with polystyrene latex (Alpar et al., 1989; Jani et al., 1990), the search for biodegradable carriers is obviously crucial. So far the smallest particle used by our group has been 50 nm polystyrene latex.

Dendrimers can be synthesized with precise molecular dimensions and a great variety of surface characteristics (Denkalwalter et al., 1981; Tomalia et al., 1990; Uhrich et al., 1991; Sakthivel et al., 1998). Potential uses include molecular transport vehicles, molecular ball bearings, flow regulators in fluids, diagnostic products, artificial enzymes and vaccines (Dvornic and Tomalia, 1996). The size of the dendrimers to a large extent can be controlled in the nanometer size range. Their size and flexibility make them suitable candidates as drug carriers. We have synthesised and characterised a series of lipidic peptide dendrimers (Sakthivel et al., 1998). One of these, consisting of 16 lipidic amino groups synthesised from appropriately protected glycine, lysine and 2-amino tetra decanoic acid adopting solid phase peptide synthetic methods (Sakthivel et al., 1998), is discussed in this communication. The dendrimer was purified by HPLC and its molecular weight confirmed by a matrix assisted laser desorption ionisation (MALDI) methodology. The dendrimer was then acetylated with tritiated acetic anhydride in pyridine. The structure of the resultant dendrimer (Fig. 1) with the tritium label in the acetyl groups was also confirmed by mass spectrometry (mol. wt. = 6300). The octanol–PBS system at pH 7.4 partition coefficient of the dendrimer is 17.5 ($\log P = 1.24$).

2. Oral absorption studies

The dendrimer discussed above was administered orally to fasted female Sprague–Dawley rats (dose:14 mg/kg, 0.2 ml). The animals were euthanized by CO_2 asphyxiation, at different time intervals (3, 6 and 24 h) and after the collection of blood, all the relevant organs (the stomach, intestine, liver, spleen, kidney) were removed. The gut tissues were then washed gently with distilled water to remove unabsorbed dendrimer. All dissected tissues were weighed, homogenised and an aliquot solubilised and analysed for radioactivity.

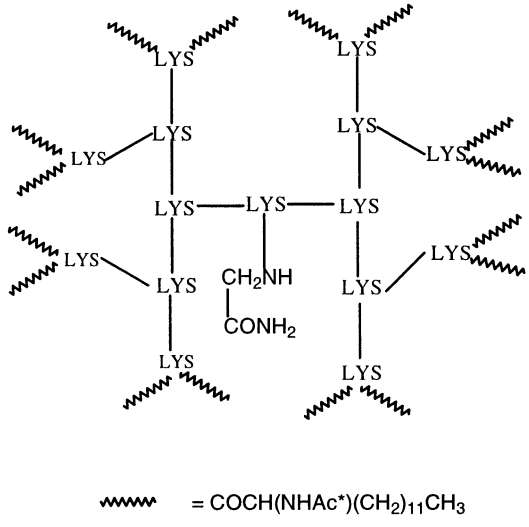

Fig. 1. Schematic diagram of the dendrimer studied.

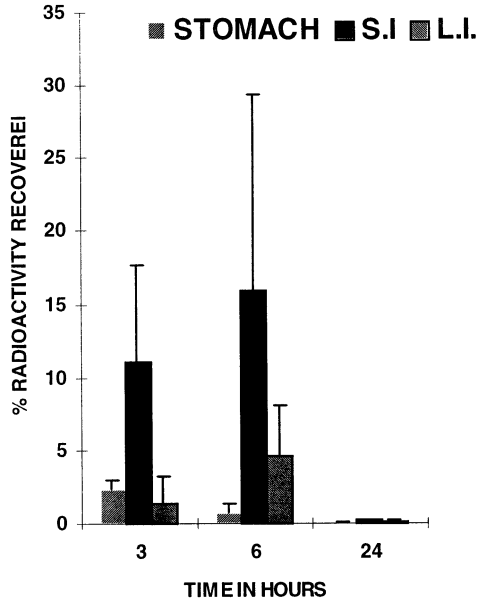

Fig. 2. Biodistribution of the dendrimer in the GI tract after oral administration.

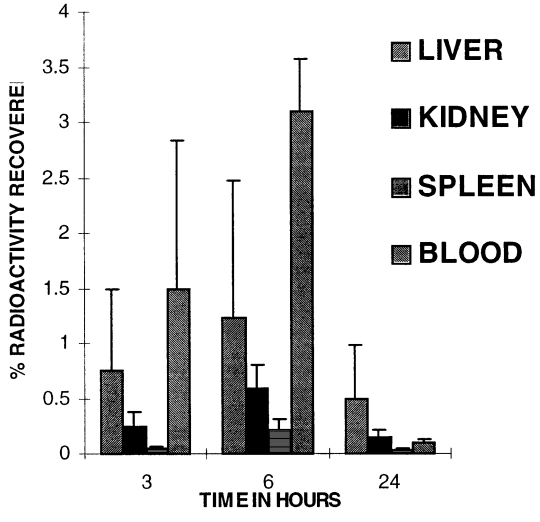

Fig. 3. Biodistribution of the dendrimer in various organs after oral administration.

For blood, the values were extrapolated to 12.8 ml for a 200 g rat (Baker et al., 1979).

The amount of dendrimer recovered after different time intervals in the stomach, small intestine and large intestine at different time intervals is given in Fig. 2. After 3 h approximately 2% of the administered dose was found in the stomach, but as at 6 and 24 h, lower levels were observed, the dendrimer migrates. In the small intestine, 15% of the administered dose was recovered after 6 h. More than 20% of the administered dose was recovered from the stomach, small intestine and large intestine after 6 h. After 24 h, less than 1% of the administered dose could be recovered, indicating clearance or absorption of the dendrimer.

The distribution of dendrimer in other organs (the liver, spleen, kidneys and blood) is shown in Fig. 3. A maximum of 1.2% was recovered in the liver and 3% in blood at 6 h, but less than 1% was found in the spleen and kidney. Maximum oral bioavailability of the dendrimer was 26.4% after 6 h, indicating rapid uptake of dendrimer from the GI tract.

3. Lymphoid uptake studies

As an extension of the study, the uptake of the dendrimer through the lymphoid and non-lymphoid tissues was measured, the dose being increased to 28 mg/kg. The results (Fig. 4) show preferential uptake (per gram tissue) of the dendrimer through the lymphoid tissue in the small intestine but not in the large intestine. The uptake through the lymphoid tissue of the small intestine was around 1% after 3 h and decreased to 0.2% after 6 h and 0.05% after 12 h. The corresponding uptake through the non-lymphoid small intestinal tissues was 3.8, 2.5 and 0.3% after 3, 6, and 12 h, respectively. In the large intestine, uptake through the lymphoid tissue of the large intestine was negligible, whereas uptake through the non-lymphoid large intestine gradually increased from 1.06% at 3 h to 3.8% at 12 h. The significance of these findings is unclear, but it can be concluded that the oral absorption of dendrimer with a diameter of the order of 2.5 nm is possible. Whether we are dealing here with molecular or particulate absorption is also not clear, but it is evident that uptake is not uniform down the gut for these 2.5 nm diameter systems.

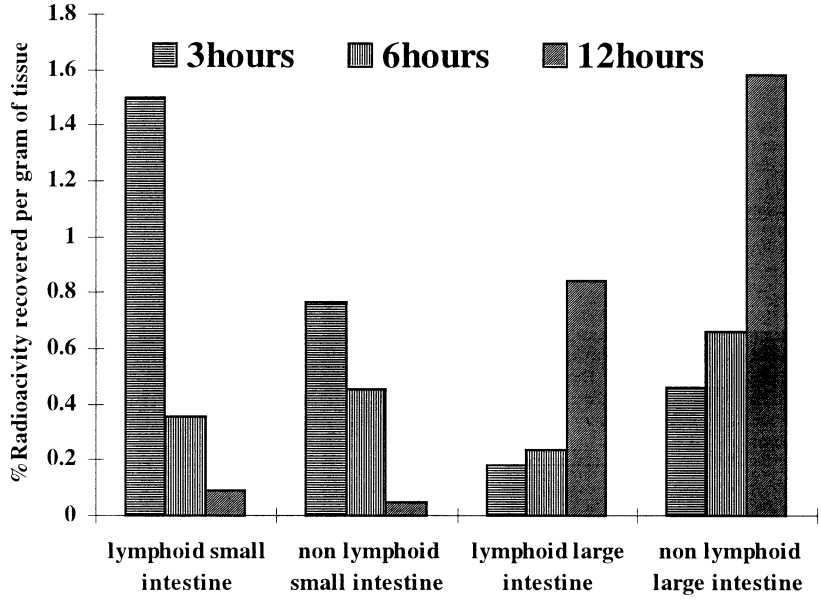

Fig. 4. Distribution of the dendrimer in the lymphoid and non-lymphoid intestine with respect to organ weight.

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