J. Pharm. Pharmacol. 1995, 47: 561–565
Received February 2, 1994
Accepted December 8, 1995

The Transfer of Polystyrene Microspheres from the Gastrointestinal Tract to the Circulation after Oral Administration in the Rat

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Abstract

Factors relating to the transfer of latex microspheres of 0·87 μm mean diameter from the gastrointestinal tract (GIT) to the circulation have been investigated.

The rapidity of appearance and the number of particles increased when the volume of water used as a suspending vehicle was increased. This was probably due to barrier cell integrity being compromised so that the movement of particles across the enterocytes would be enhanced. Particles were swept into these channels by the waterflow. The tonicity of the fluid was important as isotonic and hypertonic saline were not as affective as water in transferring particles. Particles were transferred from GIT segments adjacent to the stomach which may in part explain the rapid appearance of particles in the circulation. Particle uptake was blocked by cytochalasin B which suggests an active component may also be involved.

Despite years of controversy there is growing acceptance that colloidal particulates can both penetrate the gastrointestinal tract (GIT) and gain entry to the circulation. Opinions differ on whether particulate uptake is a significant physiological process and the wide range of mechanisms suggested to explain the process reveal the general uncertainty in the field. These include mechanisms such as persorption (Volkheimer & Schulz 1968) and endocytosis via enterocytes (Bockman & Winborn 1966) to more recently proposed mechanisms such as paracellular transport (Aprahamian et al 1987) or macrophage uptake (Wells et al 1988). Recently, several papers have concentrated on uptake through the gut-associated lympoid tissue (GALT) (Le Fevre et al 1978a, b, 1989; Jani et al 1989, 1992; Eldridge et al 1990).

Our interest in the oral uptake of colloidal particulate matter relates to the possibility of using these particles as an oral delivery system for drugs that normally have to be delivered by injection or infusion. Other possible advantages of delivery via this route using suitably modified microspheres involve the possible development of slow-release systems for drugs and protein or peptide delivery by the oral route. Also, uptake locally by GALT or by systemic immunocompetent cells after translocation of gut tissue may be useful for the production of vaccines.

Our previous work with both polystyrene and albumin particles (Lewis et al 1992) has shown that after oral dosing the uptake and transport of particles to the circulation was rapid and a high level of administered particles reached the circulation. Considerable variability in the amounts of particulate matter taken up by the GIT have been reported (O'Hagan 1990). Consequently, in this paper we have concentrated our investigation on the speed of particle uptake and also on the effect of varying the volume and tonicity of the suspending vehicle.

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Materials and Methods

Animals

All rats used were Wistar strain males, 200 g.

Anaesthesia

Anaesthesia was induced using a Boyle's veterinary anaesthetic machine to deliver 3% halothane in oxygen (300 mL min^{-1}) and nitrous oxide (1000 mL min^{-1}). Where the animals were maintained under continuous anaesthesia the halothane concentration was adjusted to 1·5% until the termination of the experiment.

Microspheres

Fluoresbrite carboxylated polystyrene latex microspheres (2·5% solids/latex) (Polysciences Ltd, Northampton, UK) with a batch diameter of 0·87 ± 0·006 μm were used in the experiments.

Direct counting of microspheres

Direct counting of particles was performed in blood samples where the blood was diluted quantitatively with glycerol and heparin (1000 units). The purpose of the glycerol was to increase the viscosity of the sample to avoid the scattering of particles to the edge of the slide. The counting was done under ultraviolet and tungsten light using a Zeiss Universal microscope and the improved Neubaur counting chamber.

Histology

Tissues immediately after collection were placed in 10% v/v formal saline (40% neutralized formaldehyde solution: 0·9% sodium chloride, 1:9) and allowed to fix for a minimum of 48 h.

Table 1. The percentage of 7×10^9 latex particles (0·87 μm diam.) in the blood after oral administration in either 0·1, 0·2, 0·3, 0·4 or 0·5 mL water. Particles were counted directly in tail-vein blood samples. Samples from three rats were counted in each group.

Water volume (mL)	Dose in the blood (%) Time (min)					
	5	15	45	120	240	250
0·1	2·1 ± 0·85	0·9 ± 0·69	2·2 ± 0·80	0·9 ± 0·4	0·4 ± 0·24	0·0 ± 0
0·2	0·87 ± 0·43	1·1 ± 0·60	0·0 ± 0	0·1 ± 0·06	0·0 ± 0	0·0 ± 0
0·3	4·1 ± 2·20	2·1 ± 1·00	2·0 ± 1·0	2·0 ± 0·71	0·4 ± 0·01	0·0 ± 0
0·4	1·1 ± 0·60	1·68 ± 0·60	1·39 ± 0·50	1·36 ± 0·65	0·5 ± 0·10	0·2 ± 0·10
0·5	5·2 ± 2·20	6·1 ± 1·16	0·4 ± 0·2	0·0 ± 0	0·0 ± 0	0·05 ± 0·03

Tissues were cut by a razor blade to 1-mm thickness and washed in alcohol and totally dehydrated in three changes of absolute alcohol over 24 h. Previous tests had shown that the ethanol had no effects on the particles and their fluorescence. Small pieces of tissue were cut using a razor blade and embedded in glycol methacrylate resin (Sims 1974) prepared by freshly mixing two solutions consisting of Reagent A (hydroxyethyl methacrylate, 90 mL; butoxyethanol, 10 mL; polyethylene glycol 400, 2 mL; and benzoyl peroxide, 1·5 g) and Reagent B (dimethylaniline). The polymerization was started by adding one drop of dimethylaniline to 10 mL reagent A and the mixture was poured over tissue sections placed in plastic moulds (Agar Scientific). Stubs were then inserted face downwards into the moulds and the resin was allowed to set for at least 2 h. The plastic moulds were removed leaving the resin-embedded section attached to the stub. Sections were cut using a Reichert-Jung microtome kindly provided by Birmingham City Hospital. Glass knives were made by a Reichert-Jung Histoknife maker which allowed sections of 500-nm thickness to be cut. These were floated on cold water and transferred to microscope slides.

The sections were stained by standard procedures with haemotoxylin and eosin and examined under a Zeiss universal microscope fitted with both tungsten and ultraviolet light sources. Particles were detected by ultraviolet light. Photographs were taken using an Olympus PM6-213181 camera.

Dosing (whole animal)

Polystyrene particles were dosed in aqueous suspension by gavage using a blunt-tipped feeding needle inserted into the stomach. Blood samples (0·1 mL) were taken from the tail vein at various times after dosing. To ensure a free flow of blood, the tail was warmed. After the sample was removed, the tail was carefully cleaned to avoid contamination of further samples. Three experiments were performed. In one the same number of particles was administered but the volume of the suspending water varied from 0·1 to 0·5 mL. In a second experiment the volume of water was fixed at 0·5 mL but the number of particles varied. In the third experiment particles were also suspended in water, 0·9% saline and 2 M saline and 0·5 mL administered by gavage to rats. Tail-vein samples were taken in each experiment as described above.

Dosing (ligated GIT segments)

The possible uptake of particles in the proximal 2-cm portion (duodenum and jejenum) of the GIT was investigated. Rats were placed under continuous anaesthesia and the abdominal cavity opened. A piece of absorbant tissue was placed under the stomach to raise it slightly and the pylorus was ligated with cotton. A dental mirror was inserted under the duodenum and mesentery, care being taken not to damage the mesentery. Slight pressure was applied to the gut with a finger to remove the gut contents and 2-cm segments including duodenum and jejenum were ligated with cotton to form sacs. Particles suspended in water were injected into the segments using a 25-gauge, syringe needle. Preliminary experiments had shown that the tissue was self-sealing after injection but as a further precaution they were made in the surface furthest from the

Table 2. The percentage 0·87 μm latex carboxylated particles in the blood after oral gavage using a range of concentrations from $1·4 \times 10^{10}$ to 7×10^{10} spheres mL^{-1}. Particles were counted directly from tail-vein samples taken individually from 3 rats in each group.

Concn spheres (mL^{-1})	Dose in the blood (%) Time (min)					
	5	15	45	120	240	250
$1·4 \times 10^{10}$	2·6 ± 1·25	4·8 ± 2·00	0·25 ± 0·16	0·4 ± 0·19	0·25 ± 0·10	0·2 ± 0·10
$2·8 \times 10^{10}$	1·0 ± 0·40	0·75 ± 0·20	0·2 ± 0·07	1·0 ± 0·18	0·1 ± 0·02	0·1 ± 0·03
$44·2 \times 10^{10}$	2·4 ± 0·83	1·5 ± 0·60	0·5 ± 0·35	0·1 ± 0·03	0·1 ± 0·04	0·05 ± 0·01
$5·6 \times 10^{10}$	1·2 ± 0·62	0·75 ± 0·23	0·6 ± 0·23	0·0 ± 0	0·0 ± 0	0·0 ± 0
$7·0 \times 10^{10}$	1·1 ± 0·34	1·75 ± 1·17	0·25 ± 0·12	0·25 ± 0·10	0·25 ± 0·01	0·0 ± 0

Table 3. The percentage of 7×10^9 0·8 μm latex particles, mixed with either water, 0·9% NaCl or 2 M NaCl, in the blood at various times after oral gavage.

	Dose in the blood (%)					
	Time (min)					
	5	15	45	120	240	250
Hypotonic	3·8 ± 0·90	5·5 ± 0·44	0·5 ± 0·10	0·5 ± 0·30	0·5 ± 0·10	0·0 ± 0
Isotonic	1·2 ± 0·70	0·6 ± 0·98	0·4 ± 0·10	0·3 ± 0·10	1·1 ± 0·52	0·5 ± 0·18
Hypertonic	1·1 ± 0·29	1·1 ± 0·60	1·1 ± 0·78	1·0 ± 0·65	1·0 ± 0·62	0·0 ± 0

mesentery. In addition, the surface was angled so that it could be rinsed with distilled water and the washings drained away from the site by aluminium foil.

In some experiments cytochalasin B (10^{-5} M) was added to the aqueous suspension before injection into the segment.

Results

Transfer of particles from the GIT to the blood and other organs

Whole animal experiments. The results shown in Table 1 show the effect of increasing the volume of water on particle uptake when the particles were administered by gavage to the stomach. The number of particles administered were 7×10^9 with a mean diameter of 0·87μm. Particles were counted directly in tail-vein blood samples. Clearly, dilution at the largest volume used (0·5 mL) increased the speed of transfer of particles to the circulation. Uptake was appreciable with up to 6% of the particles being detected in the circulation 15 min after dosing.

A similar result is indicated in Table 2. In this experiment 0·5 mL water was used in each group but the number of particles used increased from $1·4 \times 10^{10}$ to 7×10^{10}. Experimental results were identical to the results of Fig. 1. The uptake was rapid with up to 5% of the particles being present in blood 15 min after dosing.

In Table 3 the results are given where the tonicity of the suspending vehicle was varied. Three fluids were used: water (hypotonic), saline (isotonic), and saline (hypertonic). A standard volume of 0·5 mL was used for oral dosing by gavage. The results show that about 5·5% of the particles reached the circulation in 15 min after dosing with water, which is in agreement with the results shown in Tables 1 and 2. However, when isotonic or hypertonic saline was used there was a sharp fall in the numbers of particles reaching the circulation, although the presence of the particles in the blood was detected 5 min after dosing.

Isolated GIT segments. The results of varying the amount of water used as a suspending vehicle for the particles ($6·9 \times 10^9$, 0·87 μm mean diam.) are given in Table 4. Particles were counted in tail-vein samples. The results show clearly that the greatest uptake occurred when the largest volume of water was used and so supports the results found with whole animals. The results indicate that particle uptake can occur in the GIT distal to the stomach. The appearance of particles in the attached mesenteric tissue was observed 5 min after dosing.

The results in Table 5 show the effect of varying the tonicity. In all cases 0·5 mL vehicle was used but the uptake was delayed when isotonic or hypertonic saline was used, with a significant uptake being apparent at 15 min. Although these values are greater than in the corresponding water experiments, at this time the water values probably show greater losses of particles taken out of the circulation due to a longer exposure of the particles to the reticular endothelial system. The results are similar to those obtained in the whole animal (Fig. 2), when a similar pattern of uptake related to tonicity was found.

Addition of cytochalasin B to the segments

The effect of cytochalasin B is shown in Fig. 1. Cytochalasin B sharply diminished both the rate and number of particles taken up by the segments, 5 and 15 min after injection, values falling below the limit of detection after 15 min. This suggests that the uptake mechanism is an active transport process rather than a passive diffusion process.

Table 4. Uptake of microspheres from 2-cm isolated post pyloric GIT segments. The microspheres (0·87 μm mean diam.) were suspended in water (0·1–0·5 mL) and injected into the isolated segments. Particles were counted directly in tail-vein blood samples. Each result is the mean ± s.e.m. for three animals.

Volume (mL)	Microspheres in the circulation (%)
0·5	7·3 ± 2·6
0·4	1·5 ± 0·7
0·3	1·9 ± 0·3
0·2	1·0 ± 0·8
0·1	1·6 ± 1·4

Table 5. Effect of tonicity on the uptake of microspheres from 2-cm isolated GIT segments. In these experiments, the volume of the suspending vehicle was kept constant at 0·5 mL but the tonicity of the vehicle was varied. Particles were counted directly in tail-vein samples and given as mean ± s.e.m.

Vehicle	Tail-vein sample time (min)	Microspheres in circulation (%)
Water	5	15 ± 3·12
Water	15	1·0 ± 0·12
0·9% NaCl	5	<0·2 ± 0·0
0·9% NaCl	15	3·8 ± 0·5
1·8% NaCl	5	<0·2 ± 0·0
1·8% NaCl	15	1·9 ± 0·5

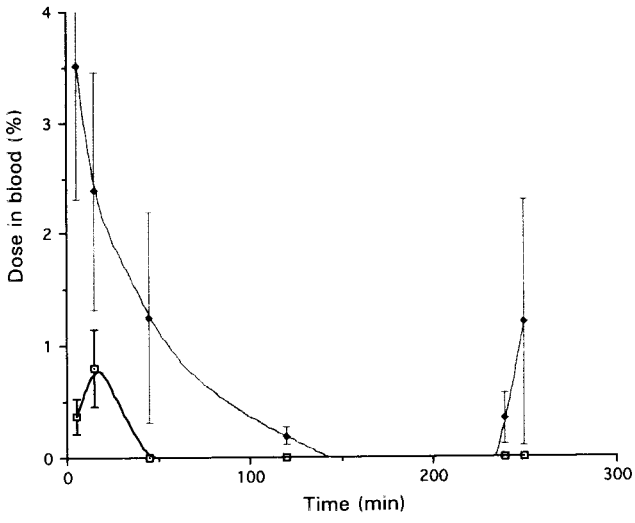

FIG. 1. Percentage of latex particles found in the circulation compartment following aqueous injection into an isolated segment of GIT in the presence ⊡ and absence ◆ of cytochalasin B. Each result is the mean ± s.e.m. of four determinations.

Histology

Figs 2 and 3 are photographs of segments taken 2 cm from the pyloric sphincter and show the effect of increasing the volume of water when dosing two rats. In Fig. 2, only 0·1 mL water was used and particles appear to have remained on the mucosal surface. In contrast, in Fig. 3, where particles were administered in 0·5 mL water, they appear in the enlarged water channels which are draining water from the mucosal to the serosal side of the GIT; these samples were taken 45 min after dosing.

Discussion

Several tentative conclusions are suggested by the results. It is clear that although particles are not transferred to the circulation via the stomach (Alpar et al 1989), they can be translocated in the GIT adjacent to the stomach and, therefore, within easy access to the stomach contents. This would explain the rapid appearance of particles in the circulation. The results have also demonstrated how the fluid environment in the GIT can affect the number and rate of particles translocated. An important factor is the surface area of the mucosae of the GIT. Due to the folds of Kerckring, villi and microvilli, the surface area cannot be regarded as that of a simple cylinder.

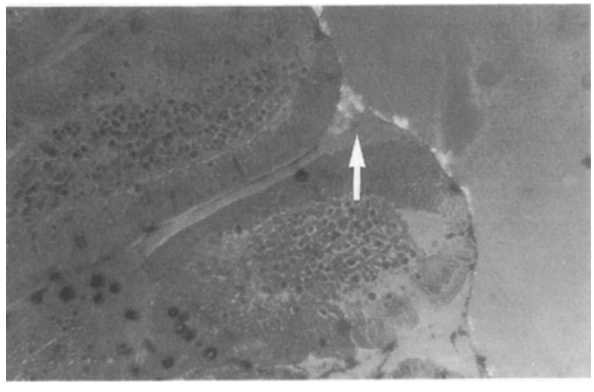

FIG. 2. Section after administration of 7×10^9 fluoresbrite carboxylate particles with 0·1 mL water (plastic section under UV × 400).

FIG. 3. Particle location after administration of 7×10^9 particles with 0·5 mL water (plastic section under UV × 400).

Data presented in Table 4 indicate the presence of a dual system of transport for particles from the GIT to the vascular compartment. Levels of particles in the blood following administration of 0·1–0·4 mL doses are similar and so transport appears to be independent of dosage volume, likely routes being the gaps at the tips of those villi where cell integration following mitosis has not been completed (Madara & Trier 1987; Volkheimer 1993), or persorption by which particles in excess of 1 μm have been shown to pass between cells (Volkheimer & Schulz 1968). It is unlikely to be associated with endocytosis which, although capable of rapidly transporting macromolecules, has not been associated with larger particles (>220 nm, Alberts et al 1989). However, the effect of cytochalasin B could indicate a phagocytic element in the already putatively complicated pattern of transfer.

The transport process appears to become volume-dependent at a dosage volume of 0·5 mL and, although more tests are necessary, it is possible that the volume is sufficient to cause expansion of the GIT lumen and so distort the lining cells. The concomitant pressure may enhance the transport through the two previously described routes or cause distortion of the enterocyte basolateral membrane which is known to expand up to 3 μm under net fluid flow (Loeschke et al 1970; Madara 1990). The water overload may also decrease the protective efficiency of the mucus barrier so enhancing the rate of penetration which would explain the results shown in Fig. 2 where particles are only observed on the surface of the mucus after the delivery of 0·1 mL of particles but in the water channels (Fig. 3) following administration of 0·5 mL of particles. The enlarged channels shown in Fig. 3 also indicates the possible effects of water overload.

The inverse correlation between vehicle tonicity and particle transport rate into the vascular compartment (Table 5) may be attributable to the reverse flow of water from the cells lining the GIT lumen into the lumen itself when the lumen contents are hypertonic to those of the

surrounding cells. This state would reduce the probability of particles presenting to the appropriate transport routes. Although it is unlikely that electrolytic influences are important in the process, experiments are in progress to assess the importance of volume and charge on the transport of these particles.

Conditions favouring the rapid uptake of particles will mean that particles will only spend a short time in the GIT. This will minimize losses of an encapsulated drug and biodegradable microspheres.

Acknowledgements

J. Eyles is grateful to the Science and Engineering Research Council for a Total Technology award supported by The Wellcome Foundation, Ltd, Dartford.

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