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The quantitation of the absorption of microparticles into the intestinal lymph of Wistar rats

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Summary

The absorption of polystyrene microparticles across the intestine of rats was examined as a preliminary assessment of the efficacy of particles as vaccine delivery systems. The superior mesenteric lymph ducts of groups ($n = 5$) of Wistar rats were cannulated and the lymph analysed by flow cytometry. This allowed direct quantification of the numbers of particles absorbed. The 0.15 and 1.0 μm particles were absorbed rapidly (after 5 min) in a temporally complex manner, with maximal absorption occurring 65 min after delivery, for both sizes of particles. Microparticles were absorbed in approximately equal numbers over 90 min (5.0×10^4 0.15 μm and 4.9×10^4 1.0 μm particles, respectively). The levels of particle absorption in the current study indicate that microparticulate delivery of vaccines may be a realistic possibility.

One of the fundamental questions concerning the applicability of microspheres as vehicles for the oral delivery of drugs and vaccines is the extent to which they are absorbed across the intestinal tract. Studies, in a variety of species, have indicated that both polystyrene and biodegradable microspheres delivered to the intestine are preferentially absorbed by the M-cells of the Peyer's patches (Jeurissen et al., 1987; Pappo and Ermak, 1989; Eldridge et al., 1990; Sass et al., 1990; Jepson et al., 1993a,b). The microparticles are believed to be delivered almost exclusively to the mesenteric lymph and systemically disseminated to a certain extent (Wells et al., 1988; O'Hagan et al., 1992).

Initial studies using biodegradable microparticles as delivery systems for the oral administration of antigens have shown elevation of the serum IgG and secretory IgA responses above those observed after the delivery of the soluble antigen (Challacombe et al., 1992). This suggests that microparticles are absorbed across the intestine to some extent. However, there are large discrepancies, between various studies, on the actual levels and temporal characteristics of microsphere absorption across the intestine, and their subsequent dissemination to other major

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organs (Le Fevre et al., 1978, 1985; Alpar et al., 1989a,b; Jani et al., 1989, 1990; Ebel, 1990). In view of the published contradictions, an accurate assessment of the levels of microparticles that are absorbed is required prior to studies to allow a realistic assessment of the potential of microparticles as drug and vaccine delivery systems.

The current communication is the first quantitative report of the levels, and temporal patterns, of microsphere absorption across the intestine to the mesenteric lymph. The study employs a cannulation procedure that ensures the collection of lymph from most of the major efferent intestinal lymphatic vessels, coupled to a highly sensitive detection system for the quantification of absorbed fluorescent microparticles. The detection methods employed for counting microparticles, in this and another recent (Ebel, 1990) study, are in contrast to the indirect methods employed previously which largely relied on the assesment of tissue latex concentrations (Alpar et al., 1989a,b; Jani et al., 1989, 1990).

Male Wistar rats (Sutton-Bonnington; 140–180 g) were fasted overnight and subsequently anaesthetized by intraperitoneal injection of sodium pentobarbitol (Sagatal, RMB Health Care Ltd, Dagenham, U.K.; 1.8 mg/kg) prior to mesenteric lymph duct cannulation based on the method of Bolman et al. (1948) utilising perspex tubing (Portex, 1.00 mm external diameter and 0.5 mm internal diameter). After implantation of the cannula, lymph was allowed to flow for 5 min prior to administration of the microparticles. Each of two groups ($n = 5$ per group) of rats received 1 ml of either 0.15 μm (approx. 1×10^{13}) or 1.0 μm (approx. 1×10^{10}) fluorescent polystyrene microparticles (Polysciences, Warrington, PA, U.S.A.) intraduodenally in the region of an observable Peyer's patch. Control animals ($n = 2$) received 1 ml saline (0.85%) intraduodenally.

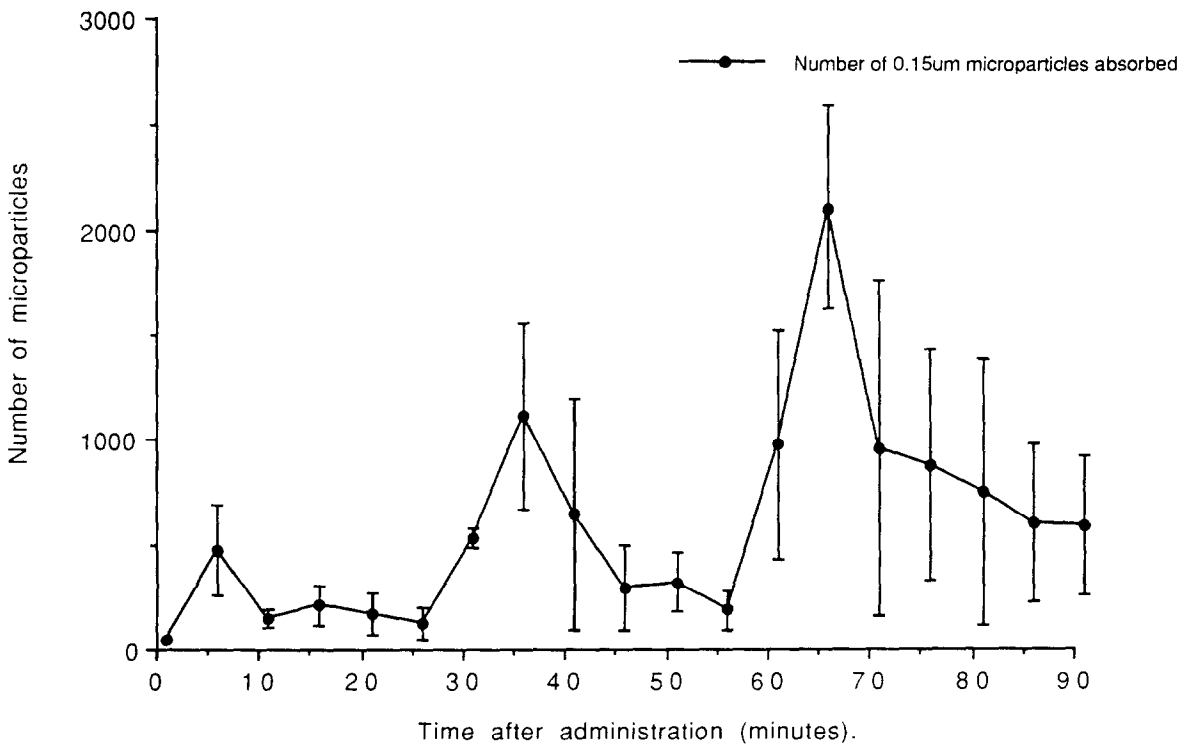

Fig. 1. The mean ($n = 5$ rats) number of 0.15 μm diameter fluorescent polystyrene microparticles absorbed into 120 μl (adjusted for dilution) of mesenteric lymph up to 90 min after intraduodenal administration. Error bars are standard errors of the mean (SE).

Mesenteric lymph was collected via the implanted cannula at 5 min intervals over 90 min and stored at 4°C for 48 h prior to flow cytometric analysis. The quantification of microparticles in the mesenteric lymph was based on modifications to the method described by Ebel (1990). Briefly, lymph samples from each individual were assayed in an EPICS V flow cytometer (Coulter Electronics Ltd, Luton, U.K.). The argon-ion laser was tuned to 457 nm wavelength, 100 mW output. Green fluorescence from the microparticles was collected by passage through a 430–490 nm band stop filter (Coulter part no. 3802072) and a 195 nm long pass filter (Coulter part no. 3802049). The threshold on the 1024 channel green ADC was adjusted to exclude the PMT noise but to provide a single peak of fluorescence. A single parameter 1024 channel histogram was collected for green fluorescence as well as a 2 parameter 128×128 channel histogram for green fluorescence correlated with time. A syringe pump (Razel, Model A99E2) was used instead of the gas pressure system which is normally employed to inject a sample into the flow cell. The sample was diluted 1:25 in 0.2 μm filtered distilled water and introduced from a 1 ml disposable plastic syringe (Plastipack, Becton-Dickinson, U.K.) via a blunt 17 G monoject needle. The syringe pump was set to deliver 20 μl/min (setting 30 on dial). 100 μl of sample was passed through the instrument before starting data collection, data was then acquired for 6 min per sample and the total number of microparticles which the EPICS detected was noted.

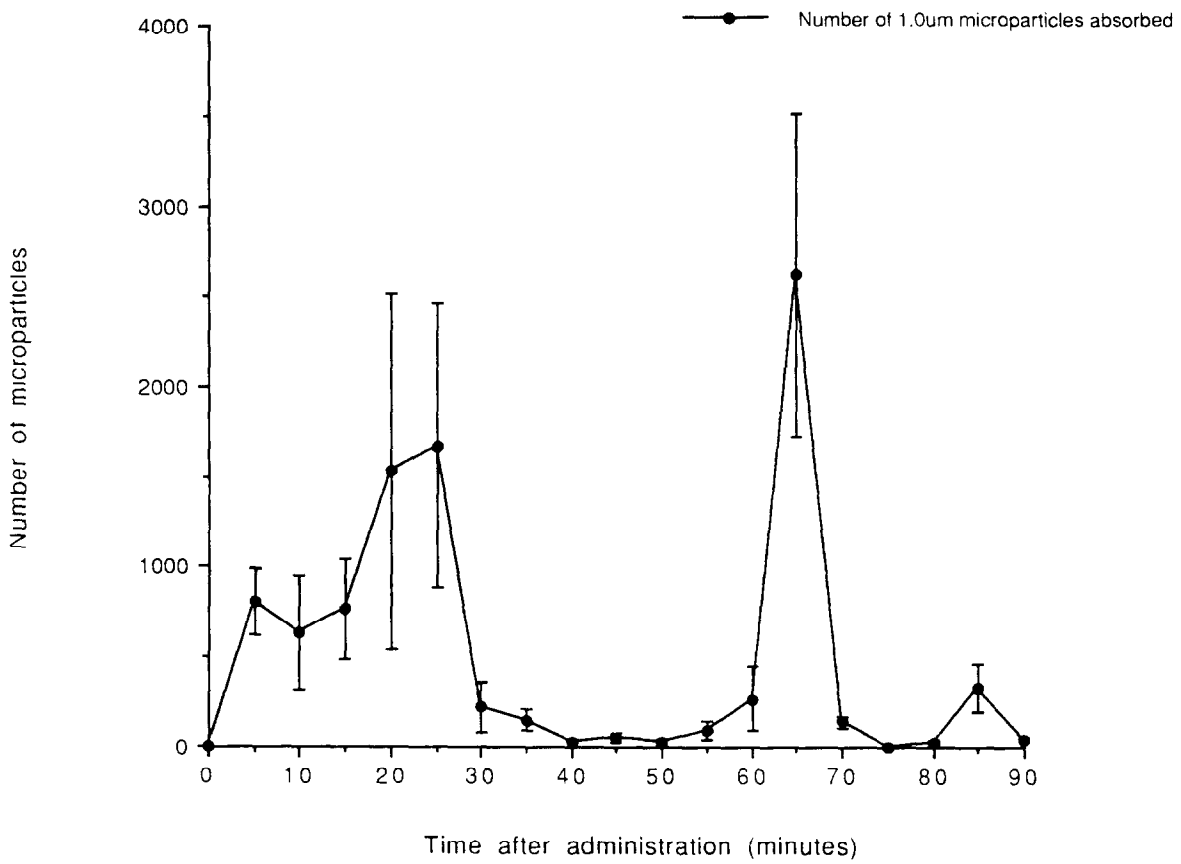

Fig. 2. The mean ($n = 5$ rats) number of 1.0 μm diameter fluorescent polystyrene microparticles absorbed into 120 μl (adjusted for dilution) of mesenteric lymph up to 90 min after intraduodenal administration. Error bars are standard errors of the mean (SE).

An extrapolated value for the number of microparticles in 120 μl of lymph was made prior to data analysis including a determination of the number and volumetric carrying capacity by an area under the curve (AUC) determination (Matthews et al., 1990), allowing a comparison of the total relative amounts of absorption of each size microsphere to be made.

The results of the flow cytometric analysis of the mesenteric lymph after delivery of both sizes of microparticles indicated that low but detectable levels of microparticles were absorbed. The administration of the 0.15 μm fluorescent microparticles revealed an apparent cyclical pattern of absorption, over 90 min, with absorptive peaks at 5, 35 and a significantly ($p = 0.01$) maximal peak at 65 min (Fig. 1). A similar pattern of absorption into the mesenteric lymph was observed after the intraduodeneal administration of 1.0 μm microparticles with peaks of absorption after 20 and 25 min and maximally after 65 min (Fig. 2). Although the two patterns of absorption appear cyclical in nature the periodicity of the absorptive events are not equal, suggesting that the sizes of the particles has some effect on at least one aspect of passage across the intestinal epithelium. Microparticles were detected as early as 5 min after intestinal administration in comparison with the findings of Sanders and Ashworth (1961), for example, where 220 nm particles were observed in the mucosal lymphatics 2 h after administration. However, this finding may merely be a reflection of the increased sensitivity of the flow cytometric assay performed here. In recent studies, microscopical analysis of particle transcytosis revealed low but detectable levels of both polystyrene, PLG particles (0.5 μm) (Jepson et al., 1993a,b) and latex beads coated with secretory IgA (Porta et al., 1992) being transcytosed across mouse M-cells after 45 min.

The levels of absorption of 0.15 and 1.0 μm particles, in the current study, were very low if assessed as a percentage of the administered dose at the time of peak absorption or throughout the time course of 90 min (Table 1). However, these levels exclude microparticles retained in the intestinal tissues and mesenteric lymph nodes and as such do not reflect total uptake. The numbers of each size microsphere absorbed over 90 min were similar, as determined by the AUC but the derived theoretical carrying capacities for the total numbers of particles delivered were substantially different (Table 1). The levels of absorption reported in the current investigation in rats, were similar to those reported previously for mice. Le Fevre et al. (1985) reported low levels of microsphere absorption from the intestine; only 0.01 and 0.0055% of single doses of 170–250 and 27 nm particles were absorbed, respectively. Ebel (1990) carried out flow cytometric analyses on various digested tissue samples, after the administration of 2.65 μm diameter microparticles orally to mice and detected approx. 0.00006% particles resident in a Peyer's patch 24 h after administra-

TABLE 1

Times and levels of peak microparticle absorption to the mesenteric lymph

	Time of maximal absorption of microparticles	Maximal number of particles absorbed (% dose)	Total number of particles absorbed to the lymph (% determined by AUC)	Theoretical volumetric carrying capacity of total number of absorbed particles (μl)
0.15 μm particles	65 min	2059 (2×10^{-8}%)	50006 (5×10^{-7}%)	0.88
1.0 μm particles	65 min	2617 (2.6×10^{-5}%)	49964 (5×10^{-4}%)	36.9

The table shows the times and levels of maximal absorption of 0.15 and 1.0 μm microparticles and also the percentage administered microparticles absorbed to the mesenteric lymph. The theoretical carrying capacity is an indirect measurement of the volume of the total number of microparticles absorbed to the lymph.

tion, 0.00003% resident in the small intestine (a 3 cm length) 48 h after administration and maximally, 0.0001% resident in the spleen, 48 h after administration.

Much larger values have been reported by Alpar et al. (1989a); 39% of a dose of 1.1 μm microparticles and 26% of 0.1 μm microparticles, given orally to rats, were absorbed after 45 min. In a related study, (Alpar et al., 1989b), approx. 37% of a dose of albumin microparticles were detected in the peripheral blood circulation 60 min after delivery. Similarly, a high level of particle absorption in rats was reported by Jani et al. (1989, 1990) where the total uptake of 1.0 μm microparticles, 2 days after the cessation of a 10 day continuous dosing regime, was 4–5%. In contrast, the levels of microparticles absorbed in the current study, as described by actual numbers absorbed or as a percentage of the administered dose (Table 1), were detectable in considerably lower numbers at the same times after delivery. The differences could be due to the occurence of other absorptive events or more likely to differences in the detection systems employed.

Recent investigations into the levels of microparticles being bound and transcytosed by rabbit Peyer's patches, using confocal microscopy, suggest that approx. 0.00005% of a dose of polylactide co-glycolide (PLG) microparticles, containing rhodamine, were absorbed 45 min after administration (Jepson et al., 1993b). Such reported levels of absorption of microparticles parallel those observed by our flow cytometric analysis of the mesenteric lymph. They also parallel electron microscopic studies of the intestinal absorption of polystyrene microparticles in Wistar rats where very few particles were detected in the M-cells (Howard et al., 1993).

The current study shows that, numerically, microparticle absorption from the intestine is low. This suggests that substantial delivery of drugs in particulate carriers across the gastrointestinal mucosa is highly unlikely but that vaccination via this route may be possible as the levels of antigen needed are often lower than those of (peptide) drugs in order to generate their respective responses. Further studies will ascertain the optimal levels of size of particle for absorption to the mesenteric lymph, the residence times of these microparticles in the Peyer's patches and mesenteric lymph nodes. Methods that can enhance the levels of microparticle absorption across the intestine will also be explored.

Acknowledgements

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