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# The transport of microspheres from the gastro-intestinal tract to inflammatory air pouches in the rat

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**Abstract**—The distribution of latex microspheres (1·1 μm diam) has been investigated in-vivo, as a potential passive targeted system for the treatment of inflammation. Microspheres administered orally were found in the circulation and in inflamed tissues and exudates of inflammatory air pouches in rats. Oral absorption was also found in a rabbit. Particles administered directly into the circulation also penetrated into the air pouch tissues and fluids. The possibility of using microspheres as a passive targeted system for the treatment of inflammation is discussed.

Microspheres are currently under investigation as potential drug carriers in-vivo and in-vitro. The use of microcarriers in-vivo poses some formidable problems yet to be resolved. A major problem is the rapid uptake by the reticuloendothelial system although this appears to have been resolved by coating hydrophobic non-biodegradable model particles prior to injection with various surfactants which impart a hydrophilic surface resistant to phagocytosis (Davis & Illum 1986). Another problem that requires to be solved if microspheres are to provide a practical means of delivering drugs in-vivo is the passage of these particles across epithelial barriers. The oral route has the obvious advantage of convenience for the administration of drugs. It has been known since 1891 following Munk's classical experiments with a patient fitted with a lymph fistula at the thigh (White et al 1964) that triglycerides could be absorbed in part appearing in the lymph as chylomicra and draining via the thoracic duct into the circulation. Volkheimer & Schultz (1968a) reported that particles from 5-150 μm could be absorbed by the intestinal tract. Since, with present technology, it is possible to prepare microspheres with diameters of a few μm, we decided to investigate oral delivery as a possible administrative route for particles. A second common route for the administration of drugs is by intraperitoneal (i.p.) injection. A recent report (DeLoach & Drolesky 1986) states that approximately 80% of erythrocytes loaded with drugs injected i.p. will reach the circulation. The remaining 20% of cells are probably removed by resident macrophages in the peritoneal cavity. The passage of erythrocytes from the peritoneal cavity to the circulation has previously been reported (Perris & Whitfield 1971). Inflammation accompanies a range of pathological disorders such as infections and rheumatic diseases. A characteristic feature of inflammation is oedema, with infiltration of leukocytes from the vascular compartment into tissues and exudates. The leukocyte count in synovial fluid from patients with rheumatoid arthritis is often used as an indicator of the severity of the underlying disease. Leukocytes enter the synovial fluid through gaps that open between epithelial cells in blood vessels under the influence of inflammatory mediators.

In this communication we have investigated the passage of latex microscopheres administered orally and i.p. to the circulation. In addition, inflammatory air pouches were examined for the presence of particles either administered orally or directly into the circulation.

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## Materials and methods

*Particles.* Latex particles (Fluoresbrite carboxylate microspheres) of various sizes were purchased from Polysciences Ltd, Northampton, UK). Latex particles were selected since this material is non-biodegradable which makes it ideal for oral administration.

*Inflammatory air-pouches.* Inflammatory air pouches were induced in male Wistar rats, 120g, by the method of Sedgwick et al (1985). Male Wistar strain rats were shaved to remove fur from the neck and back and injected subcutaneously in the scruff of the neck with 20 mL of air. Three days later the rats were each injected with a further 10 mL of air to keep the pouch inflated. A solution of carrageenan (type iv lambda, Sigma Chemical Company, Poole, Dorset) 2% m/v in physiological saline was prepared and sterilized. Two mL of this solution was injected into each air pouch six days after the initial injection of air. The inflammatory effects of the carrageenan were allowed to develop for 24 h and then particles were either injected into the heart or, in another experiment, dosed orally to the animals. The administration of particles was 24 h after that of the carrageenan. Blood samples were taken from the rats at intervals after the administration of particles. The animals were killed 24 h after the oral administration of particles and 4 h after the injection of particles into the heart. The pouch fluids were obtained by lavage and the pouches washed with two 10 mL volumes of heparinized (200 units) Hank's balanced salt solution (HBSS) containing 0·2% m/v trypsin. The pouches were incubated at 37°C for 4 h and the fluid decanted from the pouches. All fluids were centrifuged and the pellet suspended in 5 mL of saline and the particles counted in a haemocytometer using a microscope with UV illumination. The pouch lining was similarly examined after trypsin digestion for evidence of particles embedded in the surface membranes.

*Administration of particles.* Particles (1·1 μm) were administered to animals by oral administration using a plastic stomach tube. Particles were either suspended in water or in an emulsion consisting of arachis oil, water, Span 83 and Tween 80 (8:8:1:1 v/v). The volume administered was 1 mL for rats, and 5 mL for rabbits. In one experiment the emulsion included poloxamer 908 (to 0·15%).

Particles (1·1 μm) were also injected in HBSS directly into the heart (50 mL) using a Hamilton micro syringe or injected i.p. (1 mL).

*Blood samples.* Blood samples for particle and erythrocyte counts were taken from the tail vein of rodents and the ear vein of rabbits. Counts were made using a haemocytometer using a microscope with both ultraviolet and tungsten illumination (10 samples for each result).

*Ligation of the pyloric sphincter of the rat.* Two rats were anaesthetized by the i.m. injection of 0·3 mL $kg^{-1}$ 'Hypnorm' (Janssen Pharmaceutical Ltd., Oxford); and 2·5 mg $kg^{-1}$ of diazepam in 0·5 mL saline was also injected i.m. as a muscle relaxant. One rat was ligated at the pyloric sphincter with surgical cotton and the other was sham operated on where the sphincter was not ligated. The wounds were sutured and the

animals maintained in a warm condition. Each rat was dosed orally with 1 mL of water containing $1{\cdot}2 \times 10^9$, 1·1 μm latex particles in suspension. Tail vein blood samples were taken at 10 and 45 min and the number of particles present determined under UV and tungsten light using a Zeiss microscope and a haemocytometer for particle counts. The animals were not allowed to recover during the experiment and were killed after the 45 min sample was taken.

**Results**

*Transfer of particles from the gastro-intestinal tract to the circulation.* Male Wistar rats were dosed orally with $2{\cdot}05 \times 10^9$ particles mean diameter 1·1 μm. For two rats the particles were administered in water, a further two rats in emulsion and finally two rats received particles in emulsion but with Poloxamer 908 (1·5% m/v) added to the emulsion. The mean values for particles counted per 100 erythrocytes in the blood samples are given in Table 1. At 24 h only a few particles could be detected in 0·1 mL blood samples. Erythrocyte counts on the rats per $mm^3$ gave a mean value of $5{\cdot}25 \times 10^6$ cells. Since the blood volume of a 120 g rat is 8·8 mL (Archer 1965) a mean value of 1·73 particles (2.48, 0.98) per 100 erythrocytes would represent about 39% of the administered particles in circulation after 45 min in the water dosed rats. The rats that received the particles in water suspension absorbed the particles more efficiently than the rats that received the emulsion. The addition of poloxamer to the emulsion had no apparent effect on absorption.

Table 1. Transfer of particles from the gastro-intestinal tract to the circulation.

| | No of particles/100 erythrocytes Particles administered | | |
|---|---|---|---|
| Time (min) | In water | In emulsion | In emulsion but with poloxamer 908 added |
| 10 | 1·3, 0·84 | 0·32, 0·54 | 0·34, 0·30 |
| 45 | 2·48, 0·98 | 0·37, 0·45 | 0·29, 0·30 |
| 120 | 0·62, 0·47 | 0·33, 0·28 | 0·40 — |
| 180 | 0·15, 0·05 | 0·17, 0·09 | 0·02 — |

The experiment was repeated with a rabbit which received orally $9{\cdot}94 \times 10^9$ particles with a mean diameter of 0·83 μm, in water suspension. At 10 min the blood samples contained 0·16 particles per 100 erythrocytes; at 60 min 0·86 particles per 100 erythrocytes and at 2 h, 1·5 particles per 100 erythrocytes. This single rabbit was dosed to check that oral absorption occurred in species other than rat.

*Transfer of particles from the circulation to an inflammatory air pouch.* In this experiment $10^9$ particles (1·1 μm latex) were injected into the heart of 4 rats with inflammatory air pouches. The pouches were harvested 4 h after the administration of particles. In the first animal, particles were recovered from the pouch exudate representing 4·56% of the dose. About half of these particles were free and the rest engulfed by polymorphonuclear leukocytes. After trypsin treatment more particles were recovered representing 0·20% of the dose. In addition, on examination under the microscope of the upper and lower membranes in the pouch lining large numbers of particles could be seen embedded in the membranes. Although it was not possible to quantitate the numbers embedded, the average number of particles in a unit area under the microscopes were counted. In this experiment the unit area was 1·1 $mm^2$. With the first animal a mean of 13 particles in 1·1 $mm^2$ were counted in 10 fields.

The corresponding figure for a second rat for particles in the lavage fluid was 4·27% of the dose which increased by 0·02% after trypsin treatment. The average number of cells embedded in the lining was 15, per 1·1 $mm^2$. A third rat, the lavage fluid containing 0·16% of the dose, increased by 0·10 after trypsin treatment. An average of 17 particles in 1·1 $mm^2$ were embedded in the membrane. The fourth rat provided 0·22% of particles in the lavage fluid and an additional 0·01% after trypsin treatment. An average of 18 particles were embedded in the pouch lining per 1·1 $mm^2$. A tentative conclusion reached for the difference in the recovery of particles between the four rats was that aggregation of particles may have occurred in two samples causing uptake of particles in the lung. This is supported by the number of particles in circulation as determined from a tail vein blood sample taken 10 min after the injection of the particles. The figures per $mm^3$ for individual rats are 1·5 particles per 100 erythrocytes (4·76% in pouch), 1·5 particles per 100 erythrocytes (4·29% in pouch), 0·16 particles per 100 erythrocytes 0·23 erythrocytes (0·23% in pouch) and 0·09 particles per 100 erythrocytes (0·17% particles in pouch). Obviously particles were removed rapidly from the circulation of two animals. The exudates in all four pouches contained about the same number of polymorphonuclear leukocytes which indicated that the severity of inflammation was the same in all animals. The experiment demonstrated that the particles were able to reach inflamed tissues.

*Transfer of particles from the gastro-intestinal tract to the circulation and to an inflammatory airpouch.* In this experiment 50 μL portions of the commercial particle suspension ($2{\cdot}05 \times 10^9$ 1·1 μm particles) was diluted to 1 mL with water and administered to three rats or else suspended in 1 mL (of emulsion and administered to three rats by stomach tube. Blood samples taken at various times from these animals gave the results shown in Table 2. The results showed an extremely rapid oral absorption, with water being the most effective vehicle. The percentages of particles found in the pouch lavage fluids were 0·20 ± 0·05% before trypsinization for the water suspension and 0·08 ± 0·03% for the emulsion. Trypsin digestion increased the water suspension figure to 0·22% and the emulsion suspension recovery to 0·09%. A point of interest in this experiment was in the number of particles embedded in the pouch lining, in this case in a 1 $mm^2$ field. The mean density $mm^2$ for the water suspension was 78 and 117 for the emulsion. Both these values are far higher than when the pouch was harvested 4 h after the injection of particles directly into the circulation. In other work, using the same particles, we have found that polymorphonuclear leukocytes can phagocytose as many as 15 latex particles (mean diam 1·1 μm) without loss of chemotactic mobility as measured in-vitro in blind well chemotactic chambers (Hyde & Lewis—unpublished).

Table 2. Transfer of particles from the gastro intestinal tract to the circulation in the rat and to inflammatory air pouches. The number of particles in tail vein blood samples detected for 100 erythrocytes in the counting field is listed below. The time represents the interval between dosing and the removal of a blood sample. Two results are quoted for the % of the total particles found in the lavage fluids from the airpouch. These are the free particles and * free particles plus particles released after trypsin treatment.

| | No of No of particles/100 erythrocytes (Mean ± s.e.m. (3)) | | % of particles found in air pouch (mean ± s.e.m. (3)) | |
|---|---|---|---|---|
| | Particles administered | | Particles administered | |
| Time (min ) | In water | In emulsion | In water | In emulsion |
| 10 | 1·56 ± 0·33 | 0·82 ± 0·14 | — | — |
| 45 | 0·92 ± 0·15 | 0·69 ± 0·16 | — | — |
| 120 | 0·76 ± 0·18 | 0·57 ± 0·19 | — | — |
| 240 | 0·41 ± 0·11 | 0·21 ± 0·10 | — | — |
| 24 h | — | — | 0·20 ± 0·05<br>*0·22 ± 0·04 | 0·08 ± 0·03<br>*0·09 ± 0·01 |

It is possible that latex particles in the pouch wall were transported by polymorphonuclear leukocytes. It was observed that the particles were not located in blood vessels. An approximation of the number of particles embedded near the surface of the pouch lining was obtained by measuring the surface area of the pouch. The mean value was about 24 $cm^2$ which would mean that about 187 000 particles were embedded from the water suspension and 280 000 when the particles were administered in an emulsion. Since only particles close to the surface were observed it is likely that the number of particles present was much larger. Samples of rat dermis sampled away from the inflamed area did not contain particles, indicating that the deposition of particles was related to the inflammatory process.

*Delivery of intraperitoneally injected particles to the circulation.* Four rats were individually dosed i.p. with latex particles of sizes 0·8, 1·0, 4·0 and 8·0 μm. Ten minutes after dosing, the number of particles in tail vein samples were found to be 21, 24 and 26% of the number administered, respectively, for the three smaller sizes but 4% were present where 8 μm particles were administered. This experiment demonstrated that i.p. administration resulted in a fast transfer of particles to the circulation but that a cut-off point may exist for larger particles.

*Ligation of the pyloric sphincter.* No particles were detected in the blood samples taken from the rat where the pyloric sphincter had been ligated. The counts for the second pair of (sham operated) rats were: rat 1, 1·08 and rat 2, 0·82 particles per 100 erythrocytes at 10 min and rat 1, 42, rat 2, 0·56 particles per 100 erythrocytes at 45 min. The results eliminated the stomach as the site for the uptake of particles.

## Discussion

The results show that particles with an average size of 1·1 μm administered orally can penetrate into inflamed tissues and inflammatory exudates. No attempt was made to prolong the life of the particles in circulation by precoating, before administration, with agents to produce a hydrophilic surface. Previous work has involved the use the the poloxamer series of block copolymers (polyoxyethylene/polyoxypropylene) or poloxamine and natural products such as xanthans (Davis & Illum 1986). A surface coating process would probably increase the number of particles reaching inflamed tissues by maintaining a higher concentration of particles in blood over a longer period. A further possible advantage of coating would be that when the particles were eventually removed by the reticuloendothelial system the process would be gradual. Therefore, if the particles were carrying drugs the concentration of drugs in contact with RE cells would be low and toxic effects would be minimized.

A series of studies (Volkheimer 1977, Volkheimer & Schultz 1968 a, b; Volkheimer et al 1969) established that particulate matter crosses intestinal mucosa in man by 'persorption'. Volkheimer et al (1969) describes persorption as a mechanism where solid particles are 'kneaded' through the epithelial layer into subjacent tissues. He found that particles pass between the epithelial cells, especially areas of desquamation. Volkheimer (1977) found in man that particles within a size range of 7-70 μm were more readily taken up than other sizes and that mechanical factors which increased motility also increased the persorption of particles. Drugs that decreased gastric motility reduced persorption (Volkheimer & Schultz 1968a, b). It is possible that a similar mechanism may operate in the rat.

The presence of only incomplete basement membranes in epithelial lymphatic cells makes the entry of particles via the lacteals a possible route of entry from the gastrointestinal tract. The rapid appearance of particles in the circulation only 10 min after the administraton by stomach tube suggests that absorption was rapid. It was of interest that particles administered in water suspension were more rapidly taken up than those in emulsion. Water overload had been reported to increase lymph flow and the uptake of macromolecules and particles by increasing the number of gaps between epithelial lymphatic cells (Barrowman 1978). Water overload would also increase gastric motility. The results showed that absorption did not occur in the stomach. No particulate uptake by stomach has been reported (Barrowman 1978). The use of biodegradable particles by the oral route poses some problems of formulation, however, the use of enteric coated materials should allow passage of particles through the stomach. To avoid degradation in the small intestine the inclusion of protease inhibitors in the formulation would give the particles protection against serine proteases such as trypsin prior to absorption which in our experiments appears to be rapid. Alpha-1-proteinase inhibitor protects maternal antibodies from degradation from digestive proteases in colostrum.

The admission of particles to inflamed tissues will depend on the number and size of epithelial gaps. Since the size and number of these gaps will increase with the severity of inflammation, the pathology will be favourable to the entry of drug-containing particles. On resolution of the inflammation the gaps will close the access to particles will be denied.

Drug targeting allows a substantial reduction in the amount of drug that needs to be administered. It is of interest that Koff et al (1984), when studying the effect of immunomodulators encapsulated in liposomes on macrophages, found that the dose required to activate macrophages was some 800 times lower than when the free immunomodulator was used. This order of magnitude would reduce the dose of steroids in arthritis treatment to safe levels.

In this paper we have shown that orally administered particles are able to penetrate to inflamed tissues and exudates. Although formidable problems remain to be solved before this route is a practical one for the treatment of inflammatory disease we believe that these are capable of solution.

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