약제학회지(2009), 제 39 권 제 3 호
J. Kor. Pharm. Sci., Vol. 39, No. 3, 155-159 (2009)

Preliminary Imaging Analysis for Enhanced Intestinal Uptake of Non-soluble Polystyrene Microspheres in the Presence of Oleic Acid using Rat Intestine

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(Received March 25, 2009 · Revised April 3, 2009 · Accepted May 25, 2009)

ABSTRACT – In vitro intestinal uptake of non-soluble polystyrene microspheres (NPMS) was visualized with and without oleic acid using a fluorescence microscopy. Fluorescent polystyrene latex microspheres with 1 μm larger size were used as models for nonspecifically absorbed nonbiodegradable particulates. The NPMS could not penetrate the enterocytes but a few NPMS could be penetrated via Peyer's patches. When the oleic acid was mixed with NPMS, the transporting efficiency of NPMS through enterocytes as well as Peyer's patches was significantly enhanced. The modification of the intestinal membrane permeability and surface feature of the NPMS in the presence of oleic acid might be a clue to the transport of NSPM although the detailed mechanism is still under investigation.

Key words – Non-soluble polystyrene microspheres, Intestinal uptake, Oleic acid, Peyer's patch

It is now generally accepted that small amounts of inert, micro-particulate matter can breach the intestinal barrier and enter the systemic circulation.[1] Particulate delivery vehicles are largely prevented from passing between epithelial cells by tight junctions. However, since M cells possess a relatively high transcytotic capacity compared to that of enterocytes, the M cell portal may represent an efficient route for the transport of drugs and vaccines carried by particulate delivery vehicles across the intestinal epithelial barrier. Particles are absorbed by M cells present on the luminal surface of Peyer's patches; M cells are present in greatest numbers at the periphery of the epithelium covering the Peyer's patch dome. Synthetic delivery vehicles may be targeted to M cells by coating with appropriate ligands such as lectins or microbial adgesins, or the delivery vehicle may consist of a live attenuated microorganism which innately targets to M cell.[2]

The extent of microparticle absorption across the gastrointestinal tract remains a contentious issue yet it represents a basic question that must be addressed to allow a realistic assessment of the potential of microparticles as vaccine or drug vehicles. It has indicated that both polystyrene and biodegradable microspheres delivered to the intestine are preferentially absorbed by the M-cells of the Peyer's patches in a variety of species.[3] Although the particulate uptake across the gut is gaining more widespread acceptance, it is not without controversy. Part of the controversy stems from the many conflicting mechanisms that have been put forward to explain the phenomenon. Proposed mechanisms include[4] (a) persorption, (b) endocytosis by ordinary enterocytes, (c) paracellular transport, (d) uptake by intestinal macrophages and (e) uptake through the GALT.

For particulate absorption, the size is very critical for the biodistribution in the gut. The previous study also showed that the smaller polystyrene particles of 50 nm were found in both Peyer's patches and in the villi.[5,6] However, there was no evidence of the uptake of larger particles in the villi. There is now substantial evidence available that certain submicron sized particles can penetrate the GI tract.[7] A particulate colloidal carrier should have the advantages of protection from degradation, reduction of nonspecific interactions with food proteins, enhanced absorption across the intestinal epithelium and the possibililty of being targeted to the Gut Associated Lymphoid Tissue (GALT) and thus avoid the "first pass" effect of the liver.[4] Beside, most of the authors have described a simultaneous localization of small particles close to the intestinal villosities designed as Peyer's patches-free tissue.[8]

From the numerous reports, oral administration of SMEDDS incorporating lipids such as long-chain fatty acids and triglycerides may also enhance oral bioavailability of poorly bioavailable drugs by forming chylomicron with lipoprotein in intestinal tract, resulting in enhanced transport of Peyer's patches.[9-15] Thus, it is motivated whether a long chain fatty acid can modulate oral absorption of nonsoluble and non-

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biodegradable microspheres through gastrointestinal tract. Oleic acid, a common fatty acid in food was chosen as a model compound. However, there was hardly report on the effect of oleic acid on the oral uptake of particulates through intestinal cells or Peyer's patches.

In this preliminary study, the effect of oleic acid on the uptake of larger NPMS through Peyer's patches and enterocytes was visualized using fluorescent microscopic image. Fluorescent NPMS with 1 μm larger size were used as a model. NPMS is known to be nonspecifically absorbed nonbiodegradable particulates.

Materials and methods

Materials

Non-ionized and nonsoluble polystyrene microspheres (NPMS) with covalently linked fluorescein (mean diameter: 1 μm; 2.5% solid latex) were obtained from Polysciences Ltd (Northampton, UK). The NPMS size was confirmed using photon correlation spectrometry (Model Mastersizer 2000, UK). Oleic acid was purchased from Sigma (St. Louise, MO, USA).

Animal treatment

The male Sprague-Dawley rats weighting 285-380 g and aging 7-10 weeks were purchased from Daehan Biolink Co. (Chungbuk, Korea). The rats were housed with 12 h light-dark cycle (8:00-20:00) in a temperature-controlled room (25±2°C) and allowed free access to food and tap water. The rats were fasted for 24 h before the experiment but had free access to tap water.

Oral dosing and sample collection

The microspheres were administered by gavage. A dose (12.5 mg/kg) of the microsphere suspension or the microsphere suspension containing 0.1% oleic acid was given orally. The animals were given free access to water, but food was removed 10 h prior to administration of the dose. After the dose, the animals were fasted for 12 h to clear the gut of food and unabsorbed microspheres. The animals were sacrificed using the excess ether method.

After scarifying the rats, mesenteric lymph nodes and spleen were removed prior to excising the small intestine to prevent contamination of the samples with the luminal contents.[6] Peyer's patches and normal absorptive small intestine tissue that contained no Peyer's patches were removed, washed with 0.9% saline, and suspended in 0.9% saline for approximately 1 h to remove adherent particles. All dissected tissues were weighed and stored separately at -70°C before the preparation of frozen sections using a cryostat. This was preferred since some methods of traditional sectioning, fixing and cleaning of the tissue in absolute ethanol and chloroform would destroy the polystyrene microspheres.[16]

Visualization by Fluorescence microscope

The organs were maintained at -70°C using a dry ice ethanol (90%) mixture. Samples (0.5 to 1 cm^2) were taken for sectioning. To avoid cross contamination, samples from each group were sectioned and mounted on separate days. Throughout the sectioning and mounting procedures, the temperature was maintained at -30°C to -20°C. Sample tissues were embedded in a cryostat medium, and 3 μm thick sections were prepared. The sections were viewed by fluorescence microscopy.

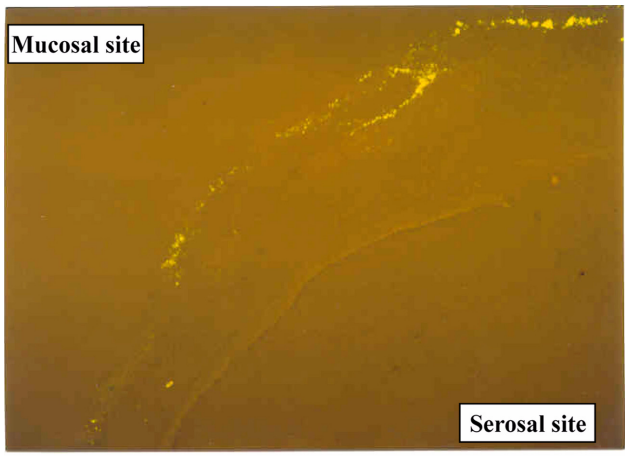
Results and discussion

The mechanism of gastrointestinal uptake of particles is still not totally understood. Three possibilities of uptake exist: an intracellular uptake, a paracellular uptake and an uptake via the M-cells, and the Peyer's patches.[7] Possibly a simultaneous uptake by more than one pathway occurs.[17] There is no doubt that uptake occurs as a natural process and not as a result of damage.[18]

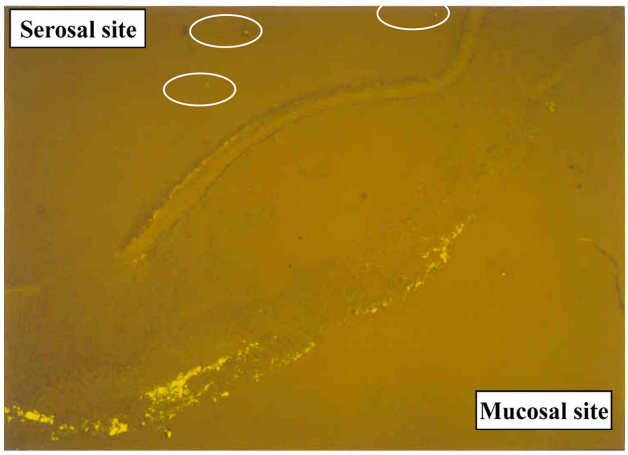
Depending on size, the particle may be retained in the Peyer's patches or transferred to the mesenteric lymph and subsequently disseminated systemically. Uptake of the polystyrene spheres is shown to be very rapid for the smaller 50 nm spheres, moderately rapid for those of 500 nm size and slow for the 1 μm beads.[6]

As can be seen in Figure 1, after a single dose of the suspension without oleic acid, some white dots of polystyrene microspheres 1 μm were found in the serosal layer of the Peyer's patches 12 h after oral administration in a small magnitude due to the poor absorption of the large latex spheres from a single dose.[6] On the contrary, the fluorescent signal of the particles was not readily detectable in the enterocytes. This indicated that 1 μm polystyrene microspheres could penetrate into Peyer's patches other than intestinal absorptive cells.

When administered with the microsphere suspension containing 0.1% oleic acid, the fluorescence image (Figure 2) showed clearly the great abundance of the polystyrene microspheres deposited in the serosal layer of the jejunum. Although recent evidence suggests that Peyer's patches are the major sites of colloidal uptake via specialized M-cells,[19] the insignificant increase in the number of the particles was also observed in the Peyer's patches. These histological observa-

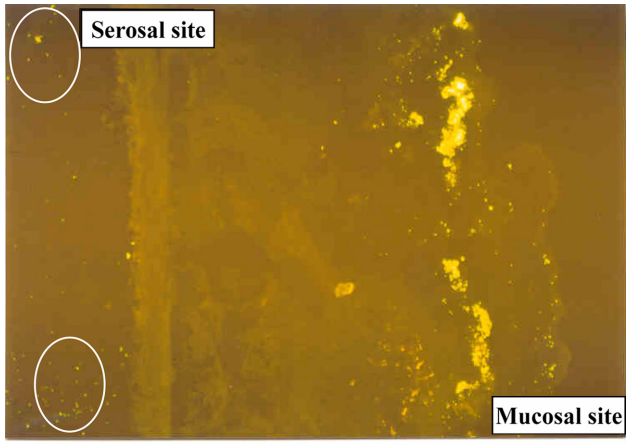


Villi, without oleic acid

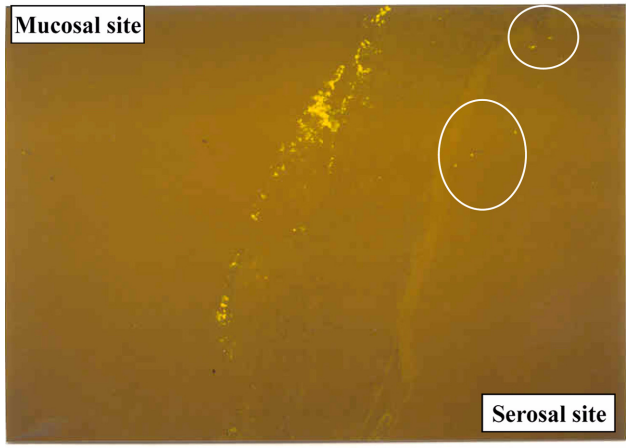


Peyer's patch, without oleic acid

Figure 1–Intestinal transport of polystyrene microspheres through villi and Peyer's patch of the rat jejunum without oleic acid.



Villi, with oleic acid



Peyer's patch, with oleic acid

Figure 2–Enhanced intestinal transport of polystyrene microspheres through villi and Peyer's patch of the rat jejunum in the presence of oleic acid.

tions indicated that the uptake of larger polystyrene microspheres does take place, not only via the M-cells in the Peyer's patches and the isolated follicles of the gut-associated lymphoid tissue, but also via the normal intestinal enterocytes when coadministered with oleic acid.

Although the gastrointestinal absorption through enterocytes is a predominant pathway to the systemic circulation, non soluble microspheres in the size range of 1 μm could penetrate through these cells for absorption without the presence of oleic acid. On the contrary, the transport of nonsoluble microspheres could be possible through Peyer's patches regardless of the presence of oleic acid. Surprisingly, the polystyrene microspheres could be transported through villi and its transporting efficiency was much pronounced when oleic acid was simultaneously combined.

Unsaturated long-chain fatty acids such as oleic acid have a prominent absorption-enhancing efficiency among natural substances.[20] The destabilization of the lipid bilayer by the incorporation of fatty acids is considered to be one of the essential processes involved in the mechanism of absorption enhancement. Similarly, Fagerlund *et al.* it indicates that fatty acids share common structural characteristics with substances that are surface-active, i.e. substances that have a hydrophobic and a hydrophilic part, can penetrate the cell membrane and thus alter the structure of the membrane and thereby increase the membrane permeability.[21] The study of Murakami *et al.* pointed that the membrane-associated SH proteins are involved in the process of the mechanism(s) of the oleic acid-induced mucosal permeability enhancement[20] by the interaction between oleic acid and the SH membrane-protein. We also reported that a semisolid self-emulsifying system (SES) of itraconazole consisting of oleic acid and polysorbate 80 was

reported to enhance the tissue uptake of itraconazole in whole intestine and Peyer's patches than that from solid dispersion. Oleic acid might form chylomicrons (80-1000 nm) within the enterocytes leading to an easy dispersion of the drug, resulting in the stimulation of transport into Peyer's patches.[15]

Florence emphasized that besides particle size the absorption process of polystyrene particles was affected by surface feature. Enhancing particle uptake was obtained when surface was hydrophobicity; several authors have discussed the possibility of targeting Peyer's patches by optimizing carrier nanoparticle surface characteristics such as hydrophobicity.[18] In this study, it was assumed that oleic acid, a hydrophobic material, modified the surface of the microsphere particles to have a proper hydrophobicity for the uptake of these particles through both pathways: enterocytes and Peyer's patches. Araujo et al. also used oil vehicles to modify the surface characteristics of polymethyl methacrylate nanoparticles of a diameter of 130 nm to improve the uptake of these nanoparticles in a single dose by oral gavage of a suspension in saline. The addition of oleic acid to the peanut oil increased the uptake of the nanoparticles by about 50%.[17]

Until now, many efforts have been made to overcome biological barriers to particulate uptake. Hussain et al. utilized bacterial mechanism for epithelial cell entry, in which polystyrene was coupled with invasin to enhance the uptake of these nonsoluble particles. In other studies, polystyrene particles were conjugated with tomato-lectin to improve its uptake through Peyer's patches, also the markedly increased uptake attained after covalent attachment to particles of tomato lectin molecules by the involvement of normal enterocytes in the uptake process.[18] In this study, simple co-administration of NPMS with oleic acid could improve uptake of these particles through enterocytes as well as Peyer's patches. This will be a promising way for enhancement of particle uptake through the intestinal barrier.

Conclusions

Based on the direct fluorescent observation of histological sections, uptake and translocation of NPMS could be varied, depending on the site and the presence of pharmaceutical excipient. Without oleic acid, a potential promoter for lymphatic targeting, NPMS was observed only in the serosal layer of the Peyer's patch without appearing in the enterocytes. In contrast, co-administration of the microsphere suspension with 0.1% oleic acid increased dramatically the number of the microspheres in the serosal layer of enterocytes. NPMS was absorbed through the Peyer's patch regardless of the presence of oleic acid. Although these data have yet to be quantified, this study confirmed an evidence for the uptake and translocation of microparticulates across the mucosal barrier.

Acknowledgment

This work was partially supported by the Korea Science and Engineering Foundation (KOSEF:R01-2008-000-11777-0), Korea. We appreciate Prof. A.T. Florence, University of London for her helpful advice and comments.

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