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Note

Biodistribution studies of BSA loaded gelatin microspheres after peroral application

K. Mladenovska [a,*], I.E. Janevik [b], D.M. Glavas [a], F.E. Kumbaradzi [a], K. Goracinova [a]

[a] *Institute of Pharmaceutical Technology, Department of Biopharmacy, Faculty of Pharmacy, University "Ss Cyril and Methodious", Vodnjanska 17, 1000 Skopje, Macedonia*

[b] *Institute of Pathophysiology and Nuclear Medicine, Faculty of Medicine, University "Ss Cyril and Methodious", Vodnjanska 17, 1000 Skopje, Macedonia*

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Abstract

Biodistribution studies of radiolabelled [^{131}I]BSA loaded gelatin microspheres were carried out on BALB/c mice after peroral administration. To two groups, the radiolabelled [^{131}I]BSA gelatin microspheres with different mean particle size, 1.196 ± 1.961 and 7.028 ± 1.231 μm were administered orally. To the control group, a solution of [^{131}I]BSA was also orally administered. Biodistribution was followed periodically within 15 days as a percent of total radioactivity present in stomach, small intestine with Peyer's patches and mesentery, colon with Peyer's patches, appendix and mesentery, liver, spleen, blood, kidney, lungs and heart. The biodistribution data confirmed that uptake in mice into Peyer's patches and passage to the liver and spleen via the mesentery lymph supply and nodes, increased with decreasing particle size.

Keywords: Gelatin microspheres; BSA; Peroral administration; Biodistribution

There is a body of evidence which suggests that the absorption of orally administered antigen in the intestine stimulates particular cells in the gut-associated lymphoid tissue, specifically T helper cells and IgA precursor B cells, and especially in the Peyer's patches, leading to dissemination of B and T cells to mucosal effector tissues for subsequent antigen-specific secretory IgA responses (Alpar et al., 2000; Trolle et al., 1998). A number of potential delivery systems, including sustained-antigen releasing microparticulated carriers have been used for targeting the Peyer's patches and protecting the antigen of interest from the harsh environment of the GIT. Although intracellular uptake, intracellular/paracellular uptake and uptake via the M-cells and Peyer's patches are the three possible mechanisms of gastro-intestinal uptake of intact particles, the majority pathway seems to be via the M-cells and Peyer's patches. (Kreuter, 1994). Further evidence for the impor-

* Corresponding author

E-mail address: kmladenovska@yahoo.com (K. Mladenovska).

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tance of Peyer's patches showed uptake restricted to particles below 10 μm in size. The microspheres with diameter 5–10 μm remained in the Peyer's patches, inducing the mucosal immunity, when the microspheres below 5 μm in diameter passed through the Peyer's patches within macrophages stimulating systemic immunity. Particles of this size were found in stomach, small intestine with Peyer's patches, appendix and mesentery, liver, spleen, blood and kidneys (Jani et al., 1992). Gelatin microspheres have already shown the strong adjuvant effect by inducing IgA secretion at the genito-urinary mucosa after peroral application to ddy mice. Particle size employed was in a range from 2 to 4 μm (Nakamura et al., 1998).

Considering the above-mentioned biodistribution studies of radiolabelled [^{131}I]BSA loaded gelatin microspheres with different particle size were carried out after oral application to BALB/c mice. The microspheres were prepared by emulsification of aqueous solution of gelatin and radiolabelled [^{131}I]BSA into the particles in oil (Kreuter, 1994). By variations in the process parameters the microspheres with high loading efficacy (80–95%) were prepared and particle size ranged from 1 to 7 μm. In vitro degradation and drug release studies in a presence of collagenase confirmed prolonged degradation ($\sim 90\%$ during 72 h) and a biphasic release pattern ($0.058 \leq \alpha \leq 0.321\ h^{-1}$; $0.066 \leq \beta \leq 0.078\ h^{-1}$), supporting the potential adjuvant efficacy of gelatin microspheres.

Biodistribution studies were carried out on BALB/c mice aged 6–8 weeks and weighing around 25 g maintained on a normal mouse diet. To two groups, [^{131}I]BSA gelatin microspheres with mean particle size 1 ± 1.961 μm (I) and 7 ± 1.231 μm (II), respectively, were administered orally. To the control group (III), solution of [^{131}I]BSA was also orally administered. Biodistribution was followed periodically within 15 days as a percent of total radioactivity present in stomach, small intestine with Peyer's patches and mesentery, colon with Peyer's patches, appendix and mesentery, liver, spleen, blood 2 ml/animal, kidney, lungs and heart. Preliminary biodistribution studies of [^{131}I]solution after oral administration to a group of mice (IV), confirmed the accumulation of ^{131}I at the thyroid ($\approx 99.00\%$) in all periods of study ($n = 3$), so the eventual radioactivity per organs and in the central circulation due to ^{131}I only, was eliminated.

The first day of administration to group I-BALB/c mice ($n = 3$), 34.98 ($\pm$ 2.10)% from total radioactivity was detected in stomach, small intestine with Peyer's patches and mesentery. The percent of radioactivity appearing in the same region during the 1st day at group II was 47.12 ($\pm$ 3.56)%. Comparatively, the percentages of total radioactivity present in other organs, separately and in the central circulation also, at both groups studied are relatively similar and with low values, except in the lungs. The high percent of radioactivity detected in lungs could be explained mainly by the biodistribution of microspheres with smaller mean diameter. Namely, after their uptake into Peyer's patches and passage via the mesentery lymph supply and lymph nodes to the lymph of thoracic duct, they traverse through the central circulation to the organs that are characterized by dense populations of macrophages, such as bronchial lumens, where they traverse into the alveoli after being phagocytized by macrophages. After that they travel up the bronchial tree and are then swallowed with the saliva and excreted with the feces or may be absorbed from the gut (Kreuter, 1994; Stites et al., 1997). The spleen uptake at group I during the 2nd day of biodistribution increased to 26.06 ($\pm$ 6.67)%, expressed as a percent of total radioactivity. In the same time, at group II, the percent of total radioactivity in the spleen was 8.46 ($\pm$ 0.99)%. The data related to the application of radiolabelled BSA-loaded gelatin microspheres at group II suggested fixation of microspheres larger than 5 μm at the Peyer's patches and mesentery in small intestine; the percent of total radioactivity in this region together with stomach was 46.16 ($\pm$ 7.03)% at group II versus 21.12 ($\pm$ 3.34)% at group I. The same tendency for accumulation into the small intestine with Peyer's patches and mesentery at group II, and into the spleen at group I was observed also, on the 4th day of application (Fig. 1a).

No significant changes in the amount of radioactivity present in small intestine with Peyer's

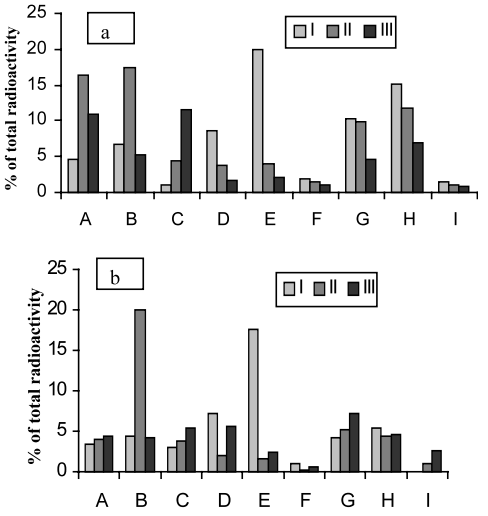

Fig. 1. Biodistribution of [^{131}I]BSA solution and [^{131}I]BSA loaded gelatin microspheres, 4 days (a) and 7 days (b) after peroral application to BALB/c mice (stomach, A; small intestine with Peyer's patches and mesentery, B; colon with Peyer's patches, appendix and mesentery, C; liver, D; spleen, E; blood 2 ml/animal, F; kidney, G; lungs, H; heart, I).

patches and mesentery (group II), and spleen (group I) were noticed on the 7th (Fig. 1b) and the 9th day of administration. After 15 days of administration, the percentages of radioactivity in regions concerned were up to 5.78 ($\pm$3.25)% in the spleen at group I, and 6.94 ($\pm$4.57)% in the small intestine at group II, suggesting low content of microspheres. No radioactivity was detected in other organs and central circulation, except in the lungs and kidneys where the radioactivity could be explained by excretion of microspheres degraded, as well as digested BSA.

The ability to protect mucosal surfaces, the common sites of pathogen entry, is perhaps the greatest attribute of oral approach to immunization. Biodegradation studies of cross-linked gelatin microspheres confirmed the stability in the GIT to digestive enzymes, such as collagenase. Considering the drug release studies, an initial pulse of antigen followed by a continuous delivery can provide protective and lasting immunity. Biodistribution studies confirm that uptake in mice into Peyer's patches and passage to the liver and spleen via the mesentery lymph supply and nodes increased with decreasing the particle size. Larger microspheres (e.g. 7 μm) tend to be adsorbed through the M cells into the Peyer's patches, thus providing a higher local antigen concentration with a potential to elicit local responses within this mucosal immune-inductive site.

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