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Tissue distribution of radioactivity following intranasal administration of radioactive microspheres

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Abstract

The aim of this study was to increase understanding of the kinetics of microparticle distribution and elimination following intranasal application. To do this we investigated the in-vivo distribution of radioactivity following intranasal instillation of scandium-46 labelled styrene-divinyl benzene 7-μm-diameter microspheres. Groups of BALB/c mice received 0.250 mg (47.5 kBq) particles suspended in either 50-μL or 10-μL volumes of phosphate buffered saline. The in-vivo distribution of radioactivity was influenced by the volume of liquid that was used to instil the microsphere suspension. Comparatively large (50 μL) administration vehicle volumes resulted in substantial bronchopulmonary deposition ($\sim$ 50 % of administered dose). Intranasal instillation of microspheres suspended in 10-μL volumes tended to restrict particle deposition initially to the nasal cavity. For both administration vehicle volumes tested, the radioactivity per unit mass of excised nasal-associated lymphoid tissue (NALT) was found to be consistently elevated relative to other tissues. This corroborates the findings of other workers who have previously identified NALT as an active site of microparticle accumulation following intranasal application. Elimination via the alimentary canal was the principal fate of intranasally applied radiolabelled material. No significant concentration of radioactivity within excised gut-associated lymphoid tissue (GALT) (Peyer's patches) was noted. At latter time points we observed, in mice that received the 50-μL volume particle suspension nasally, accumulation of potentially relevant quantities of radioactivity in the liver (0.3 % after 576 h) and spleen (0.04 % after 576 h). Thus, our data corroborate the notion that epithelial membranes in the lung are probably less exclusive to the entry of microparticulates into systemic compartments than are those mucosae in the gastrointestinal tract or nasopharynx. This effect may contribute to the effectiveness of pulmonary delivered antigen-loaded microparticles as humoral immunogens.

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Introduction

Microparticulate carriers have shown potential as vaccine delivery systems for mucosal immunisation (Alpar et al 1998; Eyles et al 1998). It is known that oral administration of microencapsulated antigens can evoke enteric and systemic immunological responses (O'Hagan 1996). Consensus is that a small percentage of orally administered microparticulate material is absorbed, principally, although probably not exclusively, via specialised epithelial cells termed microfold (M) cells (Florence 1997). While a substantial and expanding body of literature exists concerning the fate of microparticulate matter following oral administration, there is comparatively little information concerning microparticle distribution after intranasal application (Lemoine et al 1998). The purported existence of M cells in the nasopharyngeal duct, tonsils and bronchus-associated lymphoid tissue (BALT)

(Neutra et al 1996; Perry & Whyte 1998) supports the tenet that microparticle translocation to immunoresponsive tissues may occur following delivery of microparticles into the respiratory tract. In general agreement with this, Carr et al (1996) and Ridley Lathers et al (1998) reported the uptake of fluorescent polystyrene latex and poly(lactide-co-glycolide) microspheres into rodent nasal-associated lymphoid tissues (NALT) (Spit et al 1989) after intranasal administration.

Something often neglected in studies of intranasal immunisation is the potential for antigen to be deposited within the lower respiratory tract or gastrointestinal tract following nasal instillation. We have investigated the deposition and elimination of non-degradable microspheres in the lungs and intestines following nasal instillation in a murine model. Concomitant to this, we quantified radioactivity of selected systemic organs and body fluids, as the findings of earlier studies in our laboratories suggested that polystyrene nanospheres may access the blood compartment following nasal dosing (Alpar et al 1994).

Materials and Methods

Microspheres

Gamma-ray-emitting scandium-46 (half-life 84 days)-labelled styrene-divinyl benzene microspheres (7 μm diameter) (NEN-TRAC;NEN Life Science Products, UK), with a specific activity of 0.19 MBq mg^{-1}, were used for biodistribution studies.

Quantification of the extent of radiolabel leaching from NEN-TRAC microspheres

To investigate the stability of the scandium-46-microsphere complex, 20×0.250-mg quantities of NEN-TRAC microspheres were suspended in 0.5-mL volumes of PBS and vigorously agitated (with end-to-end shaking (Citenco Ltd, Herts, UK) at 37°C. At pre-selected time points (1, 24, 192, 408 and 576 h) the extent of radiolabel leaching from the particles was quantified in quadruplicate. To achieve this, 4 of the initial 20 microsphere suspensions were centrifuged at 16000 rev min^{-1} for 20 min and 200 μL of the resultant supernatant was removed. The radioactivity of the four supernatants, and their corresponding microparticle pellets with residual supernate, were then quantified using a 1282 Compugamma universal gamma counter (LKB WALLAC (Finland)). Percent leach was calculated from the ratio of radioactivity detected in the supernatants and microsphere pellets.

In-vivo fate of radiolabelled microspheres following intranasal administration

Experimentation strictly adhered to the 1986 Scientific Procedures Act. Female BALB/c mice (25 g, 6 weeks old) were used for in-vivo studies. Mice were lightly anaesthetised with an inhaled gaseous mixture of 3% (v/v) halothane (RMB Animal Health Ltd, UK) in oxygen (300 cm^3 min^{-1}) and nitrous oxide (100 cm^3 min^{-1}) for intranasal dosing procedures.

Before intranasal administration, particles were washed and re-suspended in fresh sterile PBS, and the exact radioactivity of the dose determined ($\sim$ 47.5 kBq). Female BALB/c mice (6–8 weeks old) were intranasally dosed (using a micro-pipette) with 0.250 mg of scandium-46 labelled styrene-divinyl benzene microspheres (7 μm diameter) (NEN-TRAC®), using either 10-μL or 50-μL volumes of PBS to deliver the particles. Groups of six mice were killed at 0.25, 4, 24, 96, 240 and 576 h after radiolabel administration by withdrawing cardiac blood samples under terminal inhalational anaesthesia. Freshly killed mice were then dissected as described below. Nasal wash samples were taken by inserting a narrow bore PTFE tube, attached at the opposite end to a 5-mL syringe via an appropriate gauge hypodermic needle, into the trachea via a small incision. A 5-mL volume of PBS was then expelled from the syringe and collected as it subsequently drained from the mouse's nostrils. Liver, spleen and lungs were then removed. The lumenal contents of the stomach, small intestine and large intestine were also harvested using 10-mL volumes of PBS. This was achieved with a 5-mL syringe fitted to an olive-tipped steel gavage needle. Along with the nasal washings, the three gastrointestinal-tract lavage samples were saved for radiolabel content analysis. Macroscopically visible Peyer's patches were dissected from sections of washed small intestine. Washed Peyer's patches, small intestine and large intestine were set aside for radiolabel content analysis. NALT were identified and removed from the washed mouse nasal passages as described by Asanuma et al (1997). Finally, the mouse's brain and snout were also removed and, as with the other tissue samples and washings, placed into pre-weighed mini-scintillation tubes and weighed. Dry weights of selected tissues (small intestine, Peyer's patches, large intestine and NALT) were also determined. The radioactivity of the washings, and various excised systemic and mucosal tissues, was determined using a gamma counter.

Statistics

Levels of statistical significance were assessed using analysis of variance and a two-tailed unpaired t-test. Significant differences were judged for $P < 0.05$.

Results

Quantification of the extent of radiolabel leaching from NEN-TRAC microspheres

After agitated incubation at 37°C in PBS over a period of 576 h, under 0.4% of the radioactivity associated with the NEN-TRAC microspheres was attributable to unbound scandium-46.

In-vivo fate of radiolabelled microspheres following intranasal administration

The tissue distribution of radioactivity, at selected time points following intranasal administration of scandium-46 labelled microspheres, is represented in Tables 1–3. Radiolabel distribution in-vivo was influenced by the volume of PBS that was used to nasally instil the radioactive particles. Nasal application of microspheres in 50-μL volumes of buffer resulted in substantial bronchopulmonary deposition of the NEN-TRAC microspheres (Table 1). Conversely, nasal instillation volumes of 10 μL resulted in significantly higher radioactivity in the nasopharyngeal regions at the 0.25- and 4-h time points. Irrespective of delivery vehicle volume, particle disappearance from the nasal cavity was rapid following intranasal administration (with an average of less than 12% of the administered dose recoverable from the nasal cavity 0.25 h after administration).

The presence of a significant proportion of the microparticle dose in gastric and small-intestinal washings, only 15 min after intranasal dosing, supports the tenet that a high percentage of microspheres are rapidly cleared from the nasal tract before gastrointestinal elimination. In general agreement with independent measurements of gastrointestinal transit times for similar materials (Brown et al 1998), significant numbers of microparticles were detected in large intestinal washings at the 4-h time point and beyond. It is perhaps noteworthy that significantly higher radioactivity was detected in large- and small-intestinal washings, at the 240- and 576-h time points, from mice that received the microparticles in 50-μL volumes of PBS ($P < 0.001$).

The mean percentage of administered radioactive dose detected per gram of dried gut samples and dry NALT, at time points following nasal administration of scandium-46 labelled microspheres is shown in Table 2. Appreciable and persistent, although variable, levels of radioactivity were associated with excised NALT. For mice dosed with 10-μL volumes of particle suspension, but less so for those animals which received microspheres suspended in 50 μL, radioactivity of NALT was appreciably greater than that recorded for small intestine ($P < 0.05$ at 0.25, 24, 96, 240 and 576 h), large intestine ($P < 0.05$ at 0.25, 96, 240 and 576 h) and Peyer's patches ($P < 0.001$ at 0.25, 240 and 576 h).

The radioactivity of systemic organs and tissues (blood, spleen, liver and brain) after intranasal administration of NEN-TRAC microspheres is shown in Table 3. Only mice dosed with 50-μL volumes of microparticle suspension showed evidence of radiolabel translocation to liver and spleen. Of those animals, significant levels of radioactivity were only detected in systemic tissues from 96 h after nasal dosing.

Discussion

Nasal delivery of NEN-TRAC microspheres to BALB/c mice in 10-μL volumes of PBS resulted in appreciable particle uptake into NALT but not the lungs, gut-associated lymphoid tissue (GALT) or systemic tissues. Potentially (immunologically) significant levels of microparticle accumulation within NALT has been reported by others (Carr et al 1996; Ridley Lathers et al 1998). Carr et al reported that 1-μm-diameter polystyrene microspheres accumulate within NALT following intranasal administration to anaesthetised rats (using 40 μL volume). Other workers have investigated NALT uptake of biodegradable carriers with antigen carrying capacity. Ridley Lathers et al (1998) demonstrated that 1.7-μm-diameter poly(lactide-co-glycolide) microspheres enter NALT following intranasal delivery to anaesthetised rats (using 40 μL volume). Contrary to the findings of these studies, Lemoine et al (1999) found that coumarin-loaded 8-μm-diameter poly(lactide-co-glycolide) microspheres failed to enter murine NALT following intranasal application (10 μL administration volume, with mice lightly anaesthetised). Such disparities underscore the proposal that particle uptake across mucosal barriers may well be influenced by the physicochemical properties of administered microspheres, species differences and other factors. In this respect, particle size (Donovan & Huang 1998) and hydrophobicity (Eldridge et al 1990) are thought to be important. Microspheres composed of polystyrene latexes are likely to be substantially more hydrophobic than particles fabricated from aliphatic polyesters of hydroxy

Table 1 Radioactivity (represented as the percentage of administered dose detected in entire wash sample or tissue compartment) detected within selected regions of the respiratory and gastrointestinal tracts after intranasal administration of scandium-46 labelled styrene-divinyl benzene 7-μm-diameter microspheres (NEN-TRAC) suspended in either 10-μL or 50-μL volumes of PBS to mice.

Time (h)	Wash/tissue	% administered radioactive dose in wash or tissue[a]		Statistical difference (*P*)
		10 μL administration volume	50 μL administration volume	
0.25	Nasal wash	11.819 ± 8.5350	2.5968 ± 1.9147	0.027
	Snout	32.642 ± 14.263	10.057 ± 3.0333	0.004
	Lung	0.1766 ± 0.2344	47.600 ± 12.117	0.001
	Stomach wash	12.025 ± 11.552	17.791 ± 9.9740	–
	Small-intestinal wash	16.394 ± 6.9073	12.108 ± 7.4696	–
	Large-intestinal wash	0.2283 ± 0.4269	0.0406 ± 0.0220	–
4	Nasal wash	1.8844 ± 1.8702	0.3943 ± 0.31025	–
	Snout	7.8331 ± 2.5604	2.3160 ± 0.83800	0.001
	Lung	0.0436 ± 0.0616	23.977 ± 14.3752	0.001
	Stomach wash	3.2109 ± 2.1698	0.5909 ± 0.2915	0.015
	Small-intestinal wash	11.954 ± 6.9437	3.2447 ± 1.1955	0.013
	Large-intestinal wash	33.657 ± 21.836	13.311 ± 6.0558	–
24	Nasal wash	0.2379 ± 0.0933	0.2701 ± 0.1726	–
	Snout	0.6541 ± 0.3426	0.7018 ± 0.1631	–
	Lung	0.0162 ± 0.0039	51.383 ± 35.874	0.001
	Stomach wash	0.3583 ± 0.3127	0.9540 ± 0.5572	0.046
	Small-intestinal wash	1.7276 ± 1.5211	2.8914 ± 1.0867	–
	Large-intestinal wash	2.0800 ± 1.3260	5.3424 ± 2.7325	0.025
96	Nasal wash	0.1345 ± 0.0723	0.0457 ± 0.0223	0.017
	Snout	0.4537 ± 0.2713	0.1982 ± 0.0868	–
	Lung	0.2788 ± 0.2659	28.787 ± 12.607	0.001
	Stomach wash	0.2307 ± 0.1266	0.1933 ± 0.0546	–
	Small-intestinal wash	1.3102 ± 1.3289	0.2545 ± 0.1880	–
	Large-intestinal wash	1.5859 ± 0.7912	0.7357 ± 0.6265	–
240	Nasal wash	0.0304 ± 0.0061	0.1021 ± 0.1911	–
	Snout	0.2156 ± 0.0834	0.2075 ± 0.1017	–
	Lung	0.0161 ± 0.0008	40.451 ± 14.594	0.001
	Stomach wash	0.0008 ± 0.0008	0.0424 ± 0.0145	0.001
	Small-intestinal wash	0.0422 ± 0.0039	0.0889 ± 0.0067	0.001
	Large-intestinal wash	0.0243 ± 0.0030	0.1569 ± 0.0352	0.001
576	Nasal wash	0.0553 ± 0.0213	0.0367 ± 0.0082	–
	Snout	0.1371 ± 0.1324	0.3785 ± 0.1738	–
	Lung	0.0890 ± 0.1670	34.071 ± 15.976	0.001
	Stomach wash	0.0240 ± 0.0039	0.0613 ± 0.0311	0.015
	Small-intestinal wash	0.0440 ± 0.0035	0.2286 ± 0.0753	0.001
	Large-intestinal wash	0.0292 ± 0.0072	0.2824 ± 0.0382	0.001

[a]Results are presented as means ± s.d., n = 6.

acids. Dogma suggests an inverse relationship between particle size, hydrophilicity and the potential for transmucosal uptake (Florence et al 1995).

Nasal delivery of NEN-TRAC microspheres to BALB/c mice in 50-μL volumes of PBS resulted in appreciable uptake of radioactivity into NALT. However, with this mode of nasal administration we also noted appreciable lung deposition of radioactive microspheres, and some evidence of transfer to systemic organs at later time points. It seems likely that lung-absorbed particles are trafficked through BALT, then draining vessels and lymph nodes, and ultimately enter the blood stream via the thoracic duct.

Tobío et al (1998) found they could detect radioactivity in the blood, lymph nodes, liver and spleens of rats from 24 h following intranasal delivery of ^{125}I-labelled tetanus toxoid encapsulated in polylactide-polyethyleneglycol nanospheres (142 nm). Rats were

Table 2 Radioactivity (represented as the percentage of administered dose detected per gram of dry tissue) of GALT, NALT and selected intestinal tissues after intranasal administration of scandium-46 labelled styrene-divinyl benzene 7-μm-diameter microspheres (NEN-TRAC) suspended in either 10-μL or 50-μL volumes of PBS to mice.

Time (h)	Tissue	% administered radioactive dose per gram of dry tissue[a]		Statistical difference (P)
		10 μL administration volume	50 μL administration volume	
0.25	Small intestine	0.5465 ± 0.8490	0.3209 ± 0.2507	–
	Peyer's patches	1.0718 ± 1.0909	3.2048 ± 6.8694	–
	Large intestine	0.0687 ± 0.1121	0.0430 ± 0.0346	–
	NALT	23.319 ± 16.064	7.5037 ± 8.8964	–
4	Small intestine	0.4273 ± 0.4367	0.0552 ± 0.0801	–
	Peyer's patches	1.9577 ± 1.6476	0.3217 ± 0.3822	–
	Large intestine	3.4918 ± 2.2524	0.9839 ± 1.3224	0.049
	NALT	5.7462 ± 7.5584	1.7266 ± 2.2590	–
24	Small intestine	0.03795 ± 0.036	0.1332 ± 0.1097	–
	Peyer's patches	0.2115 ± 0.2613	0.6172 ± 0.4207	–
	Large intestine	0.1404 ± 0.2198	0.5552 ± 0.5665	–
	NALT	0.6817 ± 0.5950	2.5425 ± 1.0724	0.004
96	Small intestine	0.0657 ± 0.1230	0.0210 ± 0.0126	–
	Peyer's patches	0.3168 ± 0.4476	0.1616 ± 0.2699	–
	Large intestine	0.1033 ± 0.1170	0.0692 ± 0.0453	–
	NALT	2.1427 ± 1.3574	0.8643 ± 1.4442	–
240	Small intestine	0	0.0274 ± 0.0139	0.001
	Peyer's patches	0.0007 ± 0.0018	0.1677 ± 0.1464	0.019
	Large intestine	0.0297 ± 0.0393	0.0150 ± 0.0159	–
	NALT	0.9691 ± 0.5001	0.7018 ± 1.0698	–
576	Small intestine	0.0133 ± 0.0149	0.2578 ± 0.0999	0.001
	Peyer's patches	0.1196 ± 0.2102	0.3898 ± 0.5171	–
	Large intestine	0.0118 ± 0.0127	0.1906 ± 0.1327	0.008
	NALT	0.4598 ± 0.5143	0.5538 ± 0.7485	–

[a]Results are presented as means ± s.d., n = 6.

lightly anaesthetised for nasal dosing and were administered the particles by instilling 80 μL into each nostril (20 μL four times in 3 min). They (Tobío et al 1998) noted significant lung deposition following intranasal application of the polylactide-polyethyleneglycol nanospheres. Unmodified 'plain' polylactide nanospheres (150 nm) loaded with ^{125}I-labelled tetanus toxoid were not deposited in the lungs following intranasal administration. This may be because different concentrations of particles were administered in each case, and hence the viscosity of the instilled suspension was probably not the same. The distribution of radioactivity 24 h after intranasal administration of 'plain' polylactide nanospheres (150 nm) loaded with ^{125}I-labelled tetanus toxoid indicated significantly less systemic transference of radiolabel. According to our findings, the reduced systemic uptake of radioactivity following intranasal delivery of ^{125}I-labelled tetanus toxoid in 'plain' polylactide nanospheres (in the study of Tobío et al) might be due to the reduced lung deposition of the plain particles (relative to the polylactide-polyethyleneglycol nanospheres) rather than any differences between the physicochemical properties of the two types of nanoparticles tested.

Within the time frame of the experiment, with the exception of particles deposited within the lungs, elimination via the alimentary canal was the fate for the vast majority of intranasally applied NEN-TRAC microspheres. Like NALT, gut-associated lymphoid tissues (GALT) (such as the Peyer's patches) are thought to accumulate microparticulate material after oral administration (O'Hagan 1996; Florence 1997). However, in this study, excised Peyer's-patch tissues were found to be no more radioactive than corresponding sections of washed small intestine ($P > 0.05$, except for the 240-h time point where $P = 0.042$) following intranasal in-

Table 3 Radioactivity (represented as the percentage of administered dose detected in entire tissue compartment) of selected systemic tissues after intranasal administration of scandium-46 labelled styrene-divinyl benzene 7-μm-diameter microspheres (NEN-TRAC) suspended in either 10-μL or 50-μL volumes of PBS to mice.

Time (h)	Tissue	% administered radioactive dose per tissue[a]		Statistical difference (*P*)
		10 μL administration volume	50 μL administration volume	
0.25	Blood	0.0039 ± 0.0094	0.0020 ± 0.0023	–
	Liver	0.0031 ± 0.0029	0.0048 ± 0.0032	–
	Spleen	0.0144 ± 0.0020	0.0151 ± 0.0010	–
	Brain	0.0151 ± 0.0222	0.0055 ± 0.0062	–
4	Blood	0	0.0133 ± 0.0200	–
	Liver	0.0008 ± 0.0013	0.0019 ± 0.0019	–
	Spleen	0.0147 ± 0.0222	0.0126 ± 0.0010	–
	Brain	0.0147 ± 0.0281	0.0002 ± 0.0004	–
24	Blood	0.0020 ± 0.0031	0.0010 ± 0.0011	–
	Liver	0	0.0015 ± 0.0009	0.003
	Spleen	0.0149 ± 0.0017	0.0142 ± 0.0016	–
	Brain	0.0114 ± 0.0261	0.0024 ± 0.0028	–
96	Blood	0.0020 ± 0.0022	0.0047 ± 0.0027	–
	Liver	0.0015 ± 0.0026	0.0259 ± 0.0123	0.001
	Spleen	0.0131 ± 0.0019	0.0136 ± 0.0013	–
	Brain	0.0008 ± 0.0018	0	–
240	Blood	0.0016 ± 0.0012	0.0240 ± 0.0033	0.017
	Liver	0.0016 ± 0.0019	0.0682 ± 0.0110	0.001
	Spleen	0.0155 ± 0.0020	0.0186 ± 0.0006	0.005
	Brain	0.0145 ± 0.0013	0.0153 ± 0.0170	–
576	Blood	0.0230 ± 0.0028	0.0408 ± 0.0111	0.001
	Liver	0.0228 ± 0.0054	0.2687 ± 0.0799	0.001
	Spleen	0.0203 ± 0.0014	0.0448 ± 0.0055	0.001
	Brain	0.0209 ± 0.0024	0.0235 ± 0.0034	–

[a]Results are presented as means ± s.d., n = 6.

stillation of NEN-TRAC microspheres. It is quite plausible that radioactivity associated with the intestinal samples was the result of adherent, not absorbed, particles. As compared with NALT, the lack of evidence for radiolabel uptake into intestinal compartments (including GALT) may be attributable to a number of factors: the comparatively large size (7 μm) of the NEN-TRAC spheres; the relatively low number of particles available for intestinal uptake – in oral dosing studies where significant particle uptake into GALT has been observed, much greater numbers of particles (1–10 μm diameter) were administered (often as multiple doses) than in this study (Jenkins et al 1994); the physical barrier to particle uptake provided by mucus and food breakdown products (O'Hagan 1996; Florence 1997); compared with NALT, lymphoidal tissue is distributed diffusely throughout the gut (Nugent et al 1998).

These biodistribution data support the thesis that a small percentage of appropriately intranasally administered microspheres may access sites that are conducive to the elicitation of mucosal and systemic immunological responses. We noted that, by employing different administration vehicle volumes, a prerequisite for significant translocation of radioactivity to systemic tissues was bronchopulmonary deposition of the microparticles. This implies that the epithelial barriers in the lungs are less exclusive to the entry of microparticulates into systemic compartments than are those mucosae in the nose or intestine. This is supported by the observation that lung delivery of particulate (or non-particulate) antigens results in superior systemic immune responses than if immunogen is not delivered to the lower respiratory tree following intranasal administration (Eyles et al 1999; McCluskie et al 2000). Thus, delivery of microparticulated vaccines to the lung may be one approach for engendering robust responses in the systemic arm of the immune system, without the need to compromise the skin barrier.

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