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Oral Delivery and Fate of Poly(lactic acid) Microsphere-encapsulated Interferon in Rats

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Abstract

In the light of previous findings which suggest that particulate material can be absorbed and thence systemically disseminated from the gastrointestinal tract, we have investigated the oral uptake and distribution of soluble and microsphere-encapsulated radiolabelled interferon-γ.

For trace-loaded (0·01% w/w interferon) microspheres, a quite different distribution of radioactivity was observed in-vivo 15 and 240 min after oral administration, in comparison with the control group which received equivalent doses of unencapsulated interferon-γ. Thyroid gland activity in control animals killed at these times was significantly higher than that detected in those rodents receiving trace amounts of microencapsulated interferon-γ ($P \leq 0{\cdot}05$). For poly(L-lactide) particles with higher interferon loadings (0·97% w/w interferon-γ) the distinction between the two experimental groups was less significant. During incubation in-vitro, the trace-loaded particles released a significantly lower percentage of interferon-γ in comparison with 0·97% w/w loaded microspheres ($P \leq 1$).

Bio-distribution data from rats treated orally with trace amounts of unencapsulated and microencapsulated interferon-γ leads us to the tentative conclusion that microencapsulation of proteins markedly affects oral uptake, and possibly post-absorption pharmacokinetic parameters also.

It has been established that small amounts of particulate matter partition into the gastrointestinal mucosa, and beyond, during transit along the alimentary canal (Kreuter 1991; Florence & Jani 1994; Jani et al 1994; Le Ray et al 1994). This has important implications for the delivery of therapeutic materials because, despite the numerous economic and logistical factors which favour oral drug administration over parenteral routes, most peptides and proteins are poorly absorbed from the gastrointestinal tract (Smith et al 1994) and it might be possible to enhance their uptake by use of microencapsulation technology.

Interferons are a complex group of proteins and glycoproteins with antiviral, immunomodulatory and differentiation activity. In man interferon-γ is produced by T lymphocytes in response to specific antigens to which they are sensitized, or to mitogens. Binding of dimeric interferon-γ to receptors induces biological responses via intracellular signalling and interaction with interferon-responsive genes (Heidemann 1992). Interferon-γ has a positive charge at neutral pH and a molecular weight of 17 kDa. The quaternary structure of the molecule suggests sensitivity to extremes of temperature ($> 56°C$) and pH ($< 4{\cdot}0$ and $> 9{\cdot}0$) (Farrar & Schreiber 1993).

In the absence of an appropriate pharmaceutical preparation containing absorption-promoting adjuvants, the systemic bioavailability of interferon-α and interferon-β from the gut or rectum is virtually nil (Bocci et al 1986). Because there are considerable obstacles to interferon absorption from the gut, conventional routes for interferon administration involve injection, normally subcutaneous (Bocci 1992). As non-parenteral routes of drug delivery are preferred, interferon-γ could, ideally, be administered orally in conjunction with carriers which could facilitate its gastrointestinal absorption.

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Poly(lactic acid) microspheres have been employed previously by our research group to deliver vaccines by the oral and nasal routes of administration (Alpar et al 1994). In other studies we have demonstrated the rapid translocation of polystyrene and albumin microspheres into the vascular compartment after oral delivery (Alpar et al 1989; Lewis et al 1992). We envisage that, in some instances, it might be possible to employ microsphere technology to enhance the oral bioavailability of some proteins such as interferon-γ. This has been investigated by orally administering poly(L-lactide) microsphere-encapsulated, radiolabelled interferon-γ to rats. As a control, another group of rodents was treated with unencapsulated radiolabelled interferon-γ in conjunction with empty microspheres.

Materials and Methods

Materials

(3-[^{125}I]Iodotyrosyl)interferon-γ (IM 202) was purchased from Amersham International (Bucks, UK). Unlabelled (cold) human interferon-γ was supplied as a gift from the Bender Company (Austria), in a buffered solution containing 10 μg mL^{-1} interferon-γ and an unspecified quantity of stabilizing proteins. Poly(L-lactide) with a molecular weight of 2000 (Resomer L104) was purchased from Boehringer Ingelheim (Germany). Poly(vinyl alcohol) (MW 13 000–23 000, 88% hydrolysed), methylcellulose (400 cps) and dichloromethane (HPLC grade) were supplied by Aldrich (Dorset, UK).

Microsphere preparation

(3-[^{125}I]Iodotyrosyl)interferon-γ was encapsulated within poly(L-lactide) microspheres by a double-emulsion solvent-evaporation method (Conway et al 1994). Two batches of particles were prepared. The first contained only very low

(trace) loadings of interferon-γ. For the second batch of spheres a higher concentration of radiolabelled interferon-γ and some unlabelled (cold) interferon-γ supplied by the Bender Company (Austria) was used in the manufacturing process to produce a product with a higher loading. It was calculated that these microspheres had a loading of approximately 1% w/w interferon-γ.

The primary emulsion (water-in-oil) was prepared from an aqueous solution containing the protein, poly(vinyl alcohol) (0·5% w/v; 1·25 mL) methylcellulose (0·05% w/v; 400 cps) and a solution (5% w/v; 5 mL) of the polymer in dichloromethane. The primary emulsion was formed by use of a Silverson homogenizer (Silverson Machines, UK) at 10 000 rev min^{-1} for 2 min. The resultant emulsion was then further emulsified into 75 mL of an aqueous solution (1·5% w/v) of poly(vinyl alcohol) for 5 min at 16 000 rev min^{-1}. This water-in-oil-in-water emulsion was stirred for up to 18 h to enable evaporation of the solvent. The particles were collected by centrifugation, washed three times with distilled water and freeze-dried. After preparation the microspheres were stored in a desiccator below 5°C before use in animal studies. The size distributions of the various microsphere preparations were determined using a Malvern Mastersizer/E (Malvern Instruments, UK).

Oral administration of trace-loaded interferon-containing microspheres to rats

Experiments were performed on male Wistar rats, 250 g. Before oral dosing rodents were starved overnight on grids. At the start of the experimental period each of 12 rats was weighed and then received poly(L-lactide) microspheres (10 mg) containing trace amounts of (3-[^{125}I]iodotyrosyl)-interferon-γ. The microspheres were suspended in water (1 mL) and analysed using a LKB Wallach (Sweden) 1282 Compugamma universal gamma counter. Having determined the radioactivity of each dose, the microsphere suspension was vortex-mixed and administered orally by use of a gavage needle. At 15 min, 4 h, 24 h and 4 days after gavage three animals were anaesthetized by inhalation of a gaseous mixture of 3% halothane in oxygen (300 cm^{-3} min^{-1}) and nitrous oxide (1000 cm^{-3} min^{-1}) administered by use of a Boyle's veterinary anaesthetic apparatus (British Oxygen Company, UK). Blood was then sampled from the portal vein and heart. All the animals were killed immediately after haemorrhage, while under terminal anaesthesia, and the liver, spleen, lung, kidney, mesentery and thyroid were removed. The small intestines were excized and the contents of the small intestinal lumen were harvested with copious amounts of isotonic saline by use of a gavage needle and 30-mL syringe. The large intestine was treated similarly. The washed small intestine was cut longitudinally and gently cleaned with a wet tissue. The Peyer's patches were located, micro-dissected and collected for analysis.

All pieces of gut tissue and all gut-wash samples were saved and collected for analysis of ^{125}I content using the LKB Wallach (Sweden) 1282 Compugamma universal gamma counter. For gut washes, the exact volume of the entire sample was recorded and two samples (5 mL) of this liquid were assayed. All tissue samples were weighed before analysis of ^{125}I content. For gut tissue samples, dry weights of each tissue sample (upper small intestine, lower small intestine, small intestinal Peyer's patches and large intestine) were also obtained. Rodents in the 1- and 4-day groups were placed in metabolic cages after dosing. The urine and faeces from these rats were collected and quantitatively analysed for ^{125}I content as described above.

A second (control) group of six rats was given trace amounts of unencapsulated (3-[^{125}I]iodotyrosyl)-interferon-γ orally in conjunction with a suspension of empty poly(L-lactide) microspheres prepared using the double emulsion method described above. The dose of unencapsulated interferon was identical with that given to the rats receiving trace-loaded microspheres. Each rat received 10 mg empty microspheres suspended in water (1 mL) containing the drug. The suspension was vortex-mixed before gavage. At 15 and 240 min after gavage animals were anaesthetized and blood samples were taken as described above. Three rats were killed at each time. Again, all tissue samples were weighed before analysis of ^{125}I content.

Oral administration of 0·97% w/w-loaded interferon-containing poly(L-lactide) microspheres to rats

A third group of six rats received microspheres (3 mg) with a higher ($\sim$1%) loading of (3-[^{125}I]iodotyrosyl)interferon-γ plus cold human interferon-γ. The interferon-γ-loaded spheres were given to each rat as a suspension in water (1 mL). Blood and tissue samples were taken 15 and 240 min after gavage. Three rats were killed at each time.

In a fourth group of six rats, unencapsulated (3-[^{125}I]iodotyrosyl)interferon-γ plus cold interferon-γ with empty microspheres (3 mg) was administered as a control. This fourth group of rodents received the same amount of radioactive drug as the animals given 3 mg of the 1%-loaded microspheres. Water (1 mL) was again used to suspend the empty particles and drug before gavage.

Results

Microsphere characteristics

The trace-loaded (3-[^{125}I]iodotyrosyl)interferon-γ-containing microspheres were found to have a mean diameter of 0·79 μm (standard deviation 0·48 μm). The loading of this first batch of microspheres with interferon was very low ($\sim$0·01% w/w). Thus each 10-mg batch of microspheres contained only $\sim$1 μg of interferon-γ. The mean activity of each 10-mg batch of microspheres before gavage was about 1700 Bq. We expected that to minimize the premature release of unencapsulated drug from the microspheres in-vivo, low loadings of interferon-γ should, ideally, be used. Higher interferon encapsulation efficiencies might result in a 'burst' effect, causing undesirable release of unencapsulated drug shortly after gavage. It was hoped that trace-loaded microspheres would not have this characteristic, enabling us to determine the biodistribution of encapsulated interferon-γ rather than free ^{125}I or unencapsulated drug.

The poly(L-lactide) microspheres prepared with a higher loading of interferon-γ, were found to have a mean diameter of 1·27 μm (standard deviation 0·52 μm). Mean loading was found to be 0·976% w/w interferon-γ. Thus each 3-mg batch of microspheres contained $\sim$ 30 μg of interferon-γ. The mean activity of each 3-mg batch of microspheres before gavage

Table 1. Release of interferon-γ (as a percentage of the total microsphere loading) from poly(L-lactide) microspheres into 20 mM phosphate buffer at pH 7.5.

Incubation time (h)	Release from microspheres loaded with 0·97% w/w interferon-γ*	Release from microspheres loaded with 0·01% w/w interferon-γ*
0·25	12·5 ± 3·7	2·5 ± 0·7
0·75	11·5 ± 4·0	3·4 ± 0·5
1·00	18·6 ± 5·0	5·5 ± 0·2
2·00	21·0 ± 5·4	6·2 ± 0·4
3·00	20·7 ± 6·3	5·6 ± 0·8

*Mean ± s.e.m. (n = 3).

was ~ 17 000 Bq. Empty microspheres prepared by a double-emulsion method were found to have a mean diameter of 1·12 ± 0·87 μm.

The in-vitro release profile of interferon-γ from both the 0·97 and 0·01% w/w-loaded poly(L-lactide) microspheres is shown in Table 1. The percentage of encapsulated protein released from the 0·97% w/w-loaded microspheres was found to be of a significantly higher order than the trace-loaded particles ($P \leq 0{\cdot}01$ when the two sets of data are compared with a two-way analysis of variance test).

Oral dosing experiments

When 1 μg of unencapsulated radiolabelled interferon-γ was administered orally to test animals a quite different distribution of radioactivity was recorded after 15 and 240 min in comparison with the rodents that received 1 μg of encapsulated drug (Tables 2 and 3). Substantial amounts (up to 8% of the administered radioactive dose) of radioactivity were detected in blood samples withdrawn from the heart 15 min after oral administration of trace-loaded poly(L-lactide) microspheres. Lower (approximately 3%) activity was recorded in systemic and portal blood samples withdrawn from rodents which received 1 μg of unencapsulated drug in combination with 10 mg of empty 1·12 μm poly(L-lactide) particles. Fifteen minutes after oral administration of 1 μg encapsulated interferon-γ, activity in the liver, lung and kidney was high. In rats which received 1 μg unencapsulated radiolabelled drug in combination with empty spheres, γ-ray emission from livers, spleens, lungs and kidneys removed after 15 min was significantly lower than from those tissues from the test group receiving 1 μg interferon-γ-loaded microspheres. Whereas the radioactive content of blood and tissue samples removed from rats which received trace-loaded microspheres was higher than that of blood and tissues removed from the control group after 15 min, thyroid activity was significantly lower ($P \leq 0{\cdot}05$). Mean thyroid accumulation after 15 min was 0·02 ± 0·03% of the administered dose in glands removed from rats given encapsulated drug, whereas in control rats the figure was 0·28 ± 0·14%. Such findings suggest that for animals which received trace-loaded microspheres it is mainly intact, trace-loaded microspheres which penetrate systemic compartments rapidly after oral delivery, rather than unencapsulated [^{125}I]interferon-γ, [^{125}I]interferon-γ fragments or free ^{125}I. As such it seems reasonable to suggest that for these animals most of the radioactivity detected in extra-intestinal organs after 15 min originated from the trace-loaded microspheres therein.

Four hours after oral administration, the activity of thyroids removed from all experimental animals was high, indicative of breakdown of interferon-γ and absorption in the form of free ^{125}I (Table 3). This means that we cannot say with certainty that radioactive emission from tissues removed after 4 h, 24 h and 4 days originated from microspheres containing interferon-γ within these biological samples. It is for this reason that data obtained after 24 and 48 h has not been tabulated in this communication. Despite this, it is still apparent that after 4 h the mean thyroid gland activity in those rodents which received 1 μg unencapsulated interferon-γ was statistically dissimilar ($P \leq 0{\cdot}01$) from that in those which received 1 μg encapsulated interferon-γ. This suggests that up to this time the absorption of unencapsulated and microencapsulated interferon-γ from the intestinal lumen was probably different. The

Table 2. Uptake of radiolabel 15 min after oral administration of unencapsulated or poly(L-lactide) microsphere-encapsulated interferon-γ.

Tissue	Uptake* after treatment with:			
	1 μg interferon-γ†		30 μg interferon-γ‡	
	Encapsulated	Unencapsulated	Encapsulated	Unencapsulated
Portal blood	1·31 ± 0·36	0·31 ± 0·07	0·88 ± 0·65	0·23 ± 0·05
Systemic blood	8·40 ± 0·22	3·32 ± 0·47	6·04 ± 4·00	3·46 ± 0·40
Liver	1·92 ± 0·08	1·06 ± 0·10	1·43 ± 0·63	1·04 ± 0·15
Spleen	0·21 ± 0·05	0·06 ± 0·03	0·22 ± 0·12	0·11 ± 0·04
Lung	0·88 ± 0·08	0·43 ± 0·05	0·81 ± 0·43	0·56 ± 0·09
Kidney	1·27 ± 0·06	0·52 ± 0·08	0·76 ± 0·48	0·43 ± 0·10
Upper small intestine	1·99 ± 0·79	2·33 ± 0·98	1·35 ± 0·95	1·34 ± 0·34
Lower small intestine	1·23 ± 0·60	0·80 ± 0·30	0·75 ± 0·36	0·43 ± 0·15
Peyer's patches	0·10 ± 0·07	0·81 ± 0·01	0·21 ± 0·04	0·06 ± 0·01
Large intestine	0·43 ± 0·09	0·14 ± 0·07	0·27 ± 0·03	0·13 ± 0·02
Small-intestinal wash	21·61 ± 19·70	6·09 ± 3·43	10·26 ± 13·93	3·10 ± 1·54
Large-intestinal wash	0·35 ± 0·22	0·16 ± 0·09	0·26 ± 0·34	0·20 ± 0·01
Mesentery	0·50 ± 0·03	0·26 ± 0·11	0·35 ± 0·27	0·12 ± 0·02
Thyroid	0·02 ± 0·02	0·27 ± 0·14	0·19 ± 0·13	0·13 ± 0·10

*Mean ± s.d., n = 3. †Low loading. ‡High loading.

Table 3. Uptake of radiolabel 240 min after oral administration of unencapsulated or poly(L-lactide) microsphere-encapsulated interferon-γ.

Tissue	Uptake* after treatment with:			
	1 μg interferon-γ†		30 μg interferon-γ‡	
	Encapsulated	Unencapsulated	Encapsulated	Unencapsulated
Portal blood	1·51 ± 1·30	1·02 ± 0·34	1·01 ± 0·55	0·25 ± 0·15
Systemic blood	5·79 ± 0·66	9·23 ± 1·11	7·15 ± 0·83	5·92 ± 0·76
Liver	1·14 ± 0·03	3·06 ± 0·50	1·65 ± 0·27	1·48 ± 0·22
Spleen	0·14 ± 0·03	0·25 ± 0·08	0·20 ± 0·03	0·23 ± 0·13
Lung	0·91 ± 0·49	1·11 ± 0·12	0·83 ± 0·21	1·17 ± 0·79
Kidney	1·38 ± 0·75	1·16 ± 0·09	0·08 ± 0·10	1·05 ± 0·41
Upper small intestine	0·46 ± 0·08	1·17 ± 0·23	0·45 ± 0·06	0·73 ± 0·16
Lower small intestine	0·51 ± 0·10	1·48 ± 0·27	0·42 ± 0·25	0·68 ± 0·58
Peyer's patches	0·07 ± 0·01	0·11 ± 0·04	0·20 ± 0·26	0·07 ± 0·03
Large intestine	0·43 ± 0·12	1·21 ± 0·61	0·54 ± 0·10	0·42 ± 0·08
Small-intestinal wash	9·00 ± 3·25	3·37 ± 0·07	4·78 ± 3·97	3·40 ± 2·21
Large-intestinal wash	3·46 ± 2·26	9·09 ± 1·13	1·55 ± 1·05	1·16 ± 0·20
Mesentery	0·68 ± 0·47	0·42 ± 0·09	0·33 ± 0·03	0·13 ± 0·06
Thyroid	1·52 ± 2·01	11·41 ± 2·96	4·64 ± 2·06	6·90 ± 0·98

*Mean ± s.d., n = 3. †Low loading. ‡High loading.

transit pattern of the encapsulated radioactive drug along the gastrointestinal tract is represented by data from the gut washes at the first two examinations (Tables 2 and 3). The urinary and faecal data indicated that most of the gavaged radioactivity was excreted in the urine (56·08 ± 12·65%) within the first 24 h. At 24 h the mean percentage of gavaged activity detected in the urine of animals given trace-loaded 0·79-μm diameter particles was 56·08 ± 12·65%. Faeces collected from the same rats up to 24 h after dosing contained 5·51 ± 0·94% of the administered activity. Urine collected for 4 days after oral administration of trace-loaded 0·79-μm diameter particles was found to contain an average of 62 ± 39·81% of the administered ^{125}I. Tracer absorption into the various regions of the alimentary canal after oral administration of poly(L-lactide) microspheres containing 1 μg interferon-γ was not uniform at the four different times examined (Table 4).

Discussion

Although it has not been investigated in this particular study, it is known that interferon-γ retains immunomodulatory activity after poly(L-lactide) microencapsulation procedures. Experiments in which *Yersinia pestis* V antigen, and V antigen and interferon-γ were formulated into poly(L-lactide) microspheres and injected intraperitoneally show that superior immunological responses were obtained if the microsphere preparations contained both the vaccine and interferon-γ (Griffin et al, unpublished). Elsewhere, extraction of interferon-β from poly(D,L-lactide-co-glycolide) implants retrieved up to five weeks after implantation in the mouse showed that the drug retained remarkably good antiviral activity (Eppstein et al 1986). In the light of such findings, we suggest that orally absorbed microsphere-encapsulated interferon-γ would have the potential to interact with interferon-γ-responsive elements.

Normally, high drug encapsulation efficiencies are favoured to ensure that a biological response is initiated after administration in-vivo. In this experiment, however, the specific activity of interferon-γ measured in antiviral units is about 1×10^7 units (mg protein)$^{-1}$ or higher. Addition of 100 units of interferon-γ (mL culture fluid)$^{-1}$ increases the tumoricidal activity of mouse peritoneal macrophages against B16-F10 melanoma from 0 to 12% (Eppstein et al 1985). In other words, interferon levels as low as 10^{-13} M are still effective and physiological concentrations of interferon in the range 1–2 units interferon kg^{-1} can initiate therapeutic effects (Bocci 1992). Therefore, all but very low loadings of interferon-γ might be theoretically capable of initiating a beneficial biological response in-vivo. As each 10 mg of 0·01% w/w-loaded microspheres contained the equivalent of ~1 μg or 10 000 units of interferon-γ, and each 3 mg of 0·97% w/w-loaded microspheres contained 300 000 units, the amount of interferon-γ within these preparations was deemed acceptable in terms of hypothetical therapeutic benefit. For example, our data suggests that approximately 192 units of interferon-γ are delivered to the rat liver after oral administration of trace-loaded microspheres. Although it would be highly inappropriate to equate this figure directly with bioavailability, one could hypothesize that the highly potent nature of this compound (Bocci 1992) dictates that this level of uptake would be sufficient to produce an effect.

Table 4. Radioactivity detected in the intestine after oral delivery of 1 μg radiolabelled interferon-γ encapsulated within poly(L-lactide) microspheres with a mean diameter of 0·79 ± 0·48 μm.

Time (min)	Uptake of administered activity by:*			
	Proximal small intestine	Distal small intestine	Large intestine	Peyer's patches
15	2·89 ± 1·28	0·95 ± 0·22	0·55 ± 0·22	1·29 ± 0·84
240	0·68 ± 0·30	0·76 ± 0·10	0·44 ± 0·08	1·16 ± 0·16
1440	0·23 ± 0·11	0·16 ± 0·08	0·03 ± 0·01	0·25 ± 0·23
5760	0·10 ± 0·04	0·15 ± 0·17	0·07 ± 0·03	0·23 ± 0·16

*Mean ± s.d. (n = 3) in each gram of dry, pre-washed intestinal sample as a percentage of the oral dose administered.

Administration of higher doses of interferon by use of trace-loaded microspheres would have involved administering unfeasibly large masses of particles. We examined the effect of increasing the dose by using microsphere preparations with much higher loadings of drug.

Oral administration of two different preparations comprising 0·97% w/w-loaded particles and 30 μg unencapsulated interferon-γ in conjunction with empty microspheres resulted in a very similar distribution of radioactivity (Tables 2 and 3). As such, it is not possible to comment with any certainty about the influence of the 0·97% w/w-loaded microsphere preparation on gastrointestinal absorption of [^{125}I]interferon-γ and thus subsequent discussion is concerned mainly with the data obtained from the rats given the trace-loaded microspheres. It seems likely that for the 0·97% w/w-loaded microspheres the premature release of [^{125}I]interferon-γ and subsequent breakdown of drug into unencapsulated ^{125}I resulted in a similar distribution pattern for both the control and treatment groups.

With the exception of increased thyroid activity, the organ distribution of radioactivity 4 h after oral administration of 1 μg encapsulated interferon-γ is very similar to that after 15 min, although the activity of the liver and spleen decreased slightly. This was not so for those rats that received 1 μg of unencapsulated [^{125}I]interferon-γ. In this control group the radioactive content of livers and spleens removed after 4 h was much higher than those of either group after both 15 min or 4 h. Whereas high thyroid activity after 4 h prevents elaboration regarding the biodistribution of orally administered trace-loaded microsphere-encapsulated interferon-γ, the significantly lower ($P \leq 0.005$) mean activity of the liver and systemic blood compared with that of the control group seems to reinforce the thesis that microencapsulation of interferon-γ might influence both absorption pharmacokinetic parameters a short time after oral dosing. This would help explain why systemic blood levels of radioactivity in control rats given 1 μg of unencapsulated drug increased from a level, after 15 min, statistically lower ($P \leq 0.005$) than those in the rats dosed with 1 μg encapsulated drug, to a level which was, after 4 h, statistically higher ($P \leq 0.025$).

It is possible that a large percentage of the urinary activity is attributable to intact [^{125}I]interferon-γ, as the protein is small enough to enter the ultrafiltrate, and the glomerular filter itself is negatively charged, which aids the filtration of cationic proteins such as interferon-γ (Bocci 1992). Faeces collected up to this time were much less active (5·51 ± 0·94%). Faeces collected over the 4-day experimental period contained as little as 13·15 ± 5·07% of the gavaged radiation, indicating that the majority of administered radiolabel was absorbed in one form or another.

As expected, much of the radioactivity was recovered from the small intestinal wash after 15 min (23 ± 20% for the trace-loaded spheres), with the remainder either absorbed or still resident in the stomach. Four hours after administration the majority of radioactivity was absorbed into the intestinal mucosa and beyond. Only 12·4 ± 5·5% was recoverable from the entire gut lumen after oral administration of trace-loaded microspheres, indicating that massive uptake of labelled material had occurred. This implies that most of the poly(L-lactide) carriers had broken down within either the stomach or intestinal lumen within 4 h of administration. As mentioned previously, this might result in the absorption of free ^{125}I from the lumen. Very low amounts of radiation (< 1·1% of the administered activity) were detected in intestinal washings after 1 and 4 days.

Fifteen minutes after gavage, high activity was detected in both the proximal and distal small intestine, although accumulation per unit mass was much higher in the proximal segments. This mirrors findings made using polystyrene microspheres, which demonstrated a propensity to partition into the proximal small intestinal mucosa very quickly after gavage (Hodges et al 1995). In the light of other reports describing the oral translocation of microspheres, it is noteworthy that uptake of radiolabel into the Peyer's patches was high (1·3 ± 0·8% (g dry patch tissue)$^{-1}$) relative to other intestinal regions after oral administration of trace-loaded spheres. Beyond 15 min after oral administration of poly(L-lactide) microspheres containing 1 μg interferon-γ, the levels of radiolabel within intestinal tissue declined steadily. A proportion of activity remained in these tissues up to 4 days after gavage. After 4 h, 24 h and 4 days the highest activity per unit mass of dry gut was detected in Peyer's patch tissue. Mesenteric tissue removed 24 and 96 h after gavage of the trace-loaded microspheres contained, on average, 0·04 and 0·02%, respectively, of the administered radioactivity. Unfortunately, the high levels of free ^{125}I in the body of test animals at this time preclude direct attribution of this activity to absorbed microspheres or interferon-γ or both. However, previous studies (Jani et al 1992; Jenkins et al 1994; Smith et al 1995) indicate that Peyer's patch insorbed microparticles would be routed through the mesentery on their way to the thoracic duct.

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