J. Pharm. Pharmacol. 1988, 40: 431-433
Communicated December 21, 1987

Time-dependent distribution and excretion of radiolabelled, semipermeable, stable magnetic microcapsules

A. C. POVEY, *D. GODENECHE, I. K. O'NEILL, *Unit of Environmental Carcinogens and Host Factors, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, * INSERM, Unité de Recherche U71, Etude Métabolique des Molécules Marquées, B.P. No. 184, 63005 Clermont-Ferrand, France*

Abstract—The time-dependent excretion and potential body retention of magnetic polyethyleneimine (PEI) microcapsules, methylated with [^{14}C]methyl iodide have been investigated after intragastric administration to mice. Gastric emptying was rapid but about 10% of the administered dose was still present in the stomach after 6 h; the number of microcapsules within the small intestine remained approximately constant over 1–6 h. Excretion of microcapsules in the faeces was virtually complete (98·7% excretion within 72 h), with small amounts of radioactivity excreted via the urine or as [^{14}C]CO_2. There was no detectable adsorption or retention of microcapsules within the body as measured by either a whole-body autographic study or by direct quantitation of tissue radioactivity.

Although most microencapsulated oral dosage forms are designed to release pharmaceuticals (Lim 1984; Poznansky & Juliano 1984) other systems have been designed to degrade and bind toxic intestinal metabolites, or blood-borne toxins (Chang 1969; Chang & Loa 1970; Gardner et al 1971; Sparks et al 1971). Using the interfacial polymerization technique pioneered by Chang et al (1966), we have developed magnetic microcapsules containing polyethyleneimine (PEI) to trap carcinogens or their metabolites within the gastrointestinal tract (Povey et al 1986). The microcapsules are designed to remain intact on passage through the gastrointestinal tract so that bound substances are shielded from the intestinal milieu and can be identified after magnetic extraction of the microcapsules from faeces. Microcapsules administered intragastrically have been recovered intact from faeces and have bound radioactivity after the administration of radiolabelled carcinogens by several routes (Povey et al 1987a,b,c).

The performance of such microcapsules in-vivo will depend upon, both physiological (gastric empyting, intestinal tract transit, etc.) and formulation (diffusion rates of intestinal metabolites through the microcapsule membrane, possible disintegration, etc) variables. As formation of DNA-damaging species can be localized, e.g. *N*-nitroso compounds within the stomach (Mirvish 1983), fecapentaenes within the colon (Wilkins & Van Tassell 1983), microcapsule binding of these compounds will be dependent upon such variables. For example, microcapsule binding of radioactivity after administration of [^{14}C]dimethylhydrazine (i.p.) was time-dependent and consistent with the trapping of a biliary metabolite (Povey et al 1987a). The distribution of microcapsules within the intestinal tract will be dependent more upon their gastric emptying than their small intestinal transit, as gastric emptying is affected by factors (e.g. dietary) that have little effect upon rapidity of transit of substances through small intestinal tract (Davis et al 1984, 1987). Within the colon, binding of reactive species will also be diet-dependent due to changes in colon content, residence times within the colon, bacterial microflora and endogenous metabolism (Freeman 1983).

Correspondence to: I. K. O'Neill, Unit of Environmental Carcinogens and Host Factors, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France.

The variation in observed numerical recovery of microcapsules (20–100%) has been attributed to the magnetic extraction technique used (Povey et al 1987a), but may also have arisen from retention within the body e.g. by adsorption within Peyer's patches (LeFevre et al 1978, 1980). While these microcapsules are of potential use in human studies for detecting DNA-damaging agents in high-risk populations, there is a possibility of their retention within the body which represents a potential hazard. The aims of the present study were to follow the passage of microcapsules through the intestinal tract to ultimate faecal excretion and to determine whether any microcapsules were absorbed by Peyer's patches and subsequently relocated into the systemic circulation.

Materials and methods

Preparation of microcapsules. Magnetic PEI microcapsules were prepared by an interfacial polymerization technique as described previously (Povey et al 1986) using PEI of 40–60 000 molecular weight. Characteristics of this batch have been described previously (Batch 24, Povey et al 1987d). The microcapsule mean diameter, obtained from a cumulative weight % plot was 39 μm (range 26–51 μm) and the microcapsule PEI core-to-membrane ratio was 4·7.

Microcapsules containing a total of 30 mg core PEI were incubated with 54 μmol [^{14}C]methyl iodide (sp. act. 55 mCi mmol^{-1} Amersham) for 92·5 h at 37°C in a 50:50 mixture of ethanol and 0·15 M NaCl. Radiolabel not bound to microcapsules was removed by repeated washing with ethanol followed by deionized water. Microcapsules were then further treated with simulated gastric acid (0·08 M HCl containing 0·03 M NaCl, pH 1·2) before a final washing in water. Aliquots of the radiolabelled microcapsules were then sonicated and the core-to-membrane ratio of bound radiolabel was determined (Povey et al 1986).

Treatment of animals. Male DBA 2 mice (age 6 weeks) obtained from Iffa-Credo, Lyon, France were given 1 mL of microcapsule suspension ($2·8 \times 10^6$ microcapsules; 83 KBq, 2·25 μCi) by the intragastric route. Animals were fasted at least 18 h before treatment.

Whole body autoradiographies were carried out according to the general procedure of Ullberg (1954). Briefly, at intervals after treatment, mice were killed by ether inhalation and frozen at −180°C in liquid nitrogen. Animals were mounted in a gel of carboxymethylcellulose and cut into sagittal sections (30 μm) in a cryostat (Slee, Type TS 260). Slices were applied on Kodak X-Omat films and exposed for a month.

For disposition studies, groups of five mice were killed by cervical dislocation at intervals up to 72 h after microcapsule administration. Various tissue samples were removed. In separate experiments, five animals were housed in metabolic cages enabling separate collection of urine and faeces or in an apparatus designed to measure expired $^{14}CO_2$.

Determination of radioactivity. Radioactivity of selected samples was determined in a liquid scintillation spectrometer (Packard, Model Tri-Carb 453) after combustion in a sample oxidizer (Packard, Model 306).

Results and discussion

Reaction of microcapsules with methyl iodide resulted in (i) the *N*-methylation of both PEI (core and membrane incorporated) and the terminal polyamide membrane amine groups and (ii) the formation of acid-labile methyl esters with carboxylic acid functions in the microcapsule membrane and core. Treatment of the [^{14}C]methylated microcapsules with simulated gastric acid released about 3–4% of the bound radiolabel. 64% of the remaining bound radiolabel was present in the microcapsule core bound to dissolved PEI, the remainder was associated with the microcapsule membrane. As previous studies have shown that microcapsules, previously [^{14}C-methyl]-labelled in-vitro with [^{14}C-methyl]methane sulphonate, were recovered intact from faeces after intragastric administration with high recovery of bound radioactivity (Povey et al 1987c), the link between this acid-resistant radiolabel and the microcapsules appeared to be stable under conditions present in the gastrointestinal tract. Hence, the number of microcapsules present was quantified by determination of radioactivity after sample oxidation rather than by direct microscopic observation with a haemocytometer.

Preliminary results (data not shown) obtained by microscopic counting of microcapsules extracted magnetically from stomach contents had suggested that no microcapsules were present in the rat stomach 2 h after administration. However, these results were not confirmed in the present study which, using a more sensitive technique, showed that a substantial proportion (ca 11%) of microcapsules were detected in the stomach 6 h after administration (Fig. 1). The data are consistent with an exponential model for gastric emptying as greater than 80% of administered microcapsules were emptied from the stomach within 1 h, with the subsequent rate over the next 5 h being much slower ($< 0{\cdot}05\%\ h^{-1}$). After 12 h, there were few microcapsules in the stomach ($< 0{\cdot}5\%$). This pattern of gastric emptying can explain why endogenous *N*-nitrosation of microcapsules by nitrite in drinking water (believed to occur within the stomach at acid pH) was observed when nitrite was administered 6 h after microcapsule administration intragastrically (O'Neill et al 1987) and the time-dependent binding of [^{14}C]dimethylhydrazine

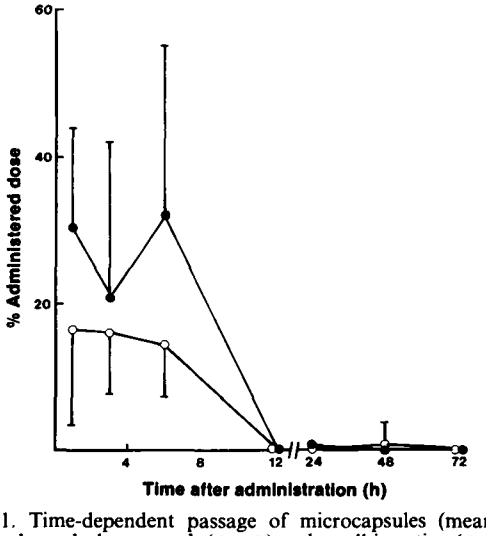

FIG. 1. Time-dependent passage of microcapsules (mean ± s.d.; n = 5), through the stomach (O—O) and small intestine (●—●).

Table 1. Cumulative excretion of radioactivity[a].

Time (h)	Faecal %	Urine %	Exhaled %
0–24	56·3	0·14	0·15
0–48	87·1	0·19	0·23
0–72	98·7	0·39	0·30

[a] Data is pooled from 5 mice administered with [^{14}C]-methylated microcapsules (5×10^6 d min^{-1} each).

metabolites (Povey et al 1987a). Previous work suggested that most microcapsules take approximately 8 h to reach the distal colon in BDVI rats (Povey et al 1987a). The present results show the number of microcapsules present within the small intestinal lumen remained approximately constant in the period 1–6 h (the large error in results arising from difficulties in obtaining the complete sample) and that after 12 h there were few remaining microcapsules ($< 0{\cdot}6\%$).

Administered radioactivity was almost all excreted ($> 98\%$) within 3 days via the faeces (Table 1). A small proportion of the administered dose ($< 1\%$) was excreted via the urine or exhaled as $^{14}CO_2$, presumably as a result of acid hydrolysis of residual [^{14}C]methyl esters, or minor leakage or radiolabelled PEI from microcapsules. Approximately 40% of administered microcapsules had not been excreted after 24 h (10% after 48 h) and were presumably present in the colon, as none were present in the upper intestinal tract (Fig. 1). A whole-body autoradiographic study (Fig. 2) indicated that radioactivity was present and confined within the intestinal tract at all periods up to 48 h. There were no levels of radioactivity detectable by autoradiography in tissues outside the gastrointestinal tract or in the upper intestinal tract 12–72 h after microcapsule administration.

The completeness of microcapsule excretion was further studied by quantifying the amount of radioactivity present in various tissues of the mouse 1–72 h after microcapsule administration. Results are presented in Table 2 as a distribution of detected radioactivity over four ranges between $< 0{\cdot}0001$ and $0{\cdot}07\%$ of administered dose mg^{-1} tissue. In most tissues (339/363; 93%), and particularly tissues outside the gastrointestinal tract (226/231; 98%), radioactivity present was very low ($< 0{\cdot}001\%$ administered dose mg^{-1} tissue). Since no localized points of radioactivity were detected by autoradiography outside the gastrointestinal tract, this was consistent with a very small escape of radioactive material from the semi-permeable microcapsules arising either from dissolved radiolabelled PEI molecules (which are otherwise retained by their molecular weight) or of incompletely eliminated acid-labile material (see Experimental). Higher levels were found in three gastrointestinal

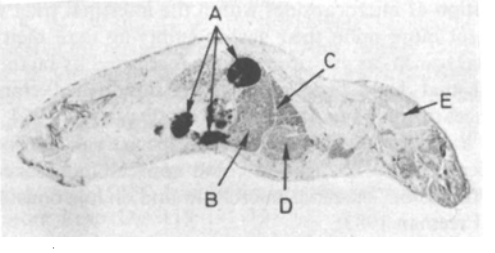

FIG. 2. Whole-body autoradiogram of DBA-2 mice 3 h after administration of [^{14}C]-methylated microcapsules. Radioactivity was found within the gastrointestinal tract (A); the tissues, liver (B), lungs (C), heart (D) and brain (E) were not radioactive and are marked solely for identification purposes.

Table 2. Distribution of radioactivity detected in mouse tissues after administration of radiolabelled microcapsules. Animals were killed 1, 3, 6, 12, 24, 48 and 72 h after microcapsule administration and tissue radioactivity quantified after sample oxidation. Groups of 5 mice were used at each time point except 24 and 48 h when 4 animals were used. Results are expressed as the total number of tissues for all the animals and time points found within the stated range.

	Range of detected radioactivity (% administered dose mg^{-1} tissue)			
Tissue	<0·0001	0·0001–0·001	0·001–0·01	0·01–0·07
Blood	33	0	0	0
Plasma	33	0	0	0
Thymus	30	3	0	0
Liver	33	0	0	0
Kidney	31	2	0	0
Spleen	32	1	0	0
Lungs	24	4	3[a]	2[b]
Stomach	16	0	13[c]	4[d]
Intestinal mucosa	25	7	1[b]	0
Peyer's patches	24	8	1[b]	0
Mesenteric lymph node	29	4	0	0

[a] Detected at 24, 48 and 72 h after microcapsule administration.
[b] At 6 h.
[c] At 1 h (2 samples); 3 h (5); 6 h (4); 24 h (1); 48 h (1).
[d] At 1 h (3 samples); 6 h (1).

tract tissues (stomach, Peyer's patches and the intestinal mucosa) but only one other non-gastrointestinal tract tissue (lungs). The distribution of stomach radioactivity was bimodal with high levels of radioactivity detected up to 60 h after microcapsule administration, and so may have occurred due to unavoidable contamination with microcapsules in residual lumenal contents (Fig. 1). Radioactivity in the lungs was found only at time points greater than 6 h; in four of the five mice (except for the animal at 72 h) higher levels (>0·001% administered dose mg^{-1} tissue) were also found in the stomach but not in any other tissue. Hence radioactivity in the lung may have been due to the initial microcapsule gavage, and subsequent slow clearance.

LeFevre et al (1978, 1980) have shown that latex microspheres may be adsorbed via the Peyer's patches in a size-dependent fashion. In the present study, there was no difference between the level of radioactivity in the intestinal mucosa and the Peyer's patches and also low levels of radioactivity (<0·001% administered dose mg^{-1} tissue) in tissues (liver, spleen) where microcapsules may be subsequently trapped (Table 2). The present data would suggest that there was no detectable adsorption of microcapsules after a single administration of radiolabelled microcapsules, and that administered microcapsules were thus larger than absorbable size, which for latex microspheres was approximately 2 μm and thus not retained in Peyer's patches (LeFevre et al 1980). Smaller, less magnetic microcapsules, had been removed previously from the batch before use by a magnetic extraction step described by Povey et al (1986).

In conclusion, these results for a single dose of administered microcapsules show them to be essentially excreted via the faeces with no detectable adsorption or retention of microcapsules within the body.

We wish to thank Mrs J. Papon for her technical assistance and Mrs M.-B. D'Arcy and Mrs M. Wrisez for preparing the manuscript. This study was supported by NIH Grant No. RO1 CA 39417-01.

References

Chang, T. M. S. (1969) Removal of endogenous and exogenous toxins by a microencapsulated absorbent. Can. J. Physiol. Pharmacol. 47: 1043–1045

Chang, T. M. S., MacIntosh, F. C., Mason, S. G. (1966) Semipermeable aqueous microcapsules. I. Preparation and properties. Can. J. Physiol. Pharmacol. 44: 115–128

Chang, T. M. S., Loa, S. K. (1970) Urea removal by urease and ammonia absorbent in the intestine. Physiologist 13: 70

Davis, S. S., Hardy, J. T., Taylor, M. J., Whalley, D. R., Wilson, C. G. (1984) The effect of food on the gastrointestinal transit of pellets and an osmotic device (Osmet). Int. J. Pharm. 21: 331–339

Davis, S. S., Khoshla, R., Wilson, C. G., Washington, N. (1987) Gastrointestinal transit of a controlled-release pellet formulation of tiaprofenic acid and the effect of food. Int. J. Pharm. 35: 253–258

Freeman, H. J. (1983) Dietary fibres and colon cancer. In Autrup, H., Williams, G.M. (eds) Experimental Colon Carcinogenesis, CRC Press Inc., Florida, pp 267–279

Gardner, D. L., Falb, R. D., Kim, B. C., Emmerling, D. C. (1971) Possible uremic detoxification via oral-ingested microcapsules. Trans. Am. Soc. Artif. Int. Organs 17: 239–245

LeFevre, M. E., Hancock, D. C., Joel, D. D. (1980) Intestinal barrier to large particulates in mice. J. Toxicol. Environ. Health, 6: 691–704

LeFevre, M. E., Olivo, R., Vanderhoff, J. W., Joel, D. D. (1978) Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes. Proc. Soc. Exp. Biol. Med. 159: 298–302

Lim, F. (1984) Biomedical Applications of Microencapsulation. CRC Press, Florida

Mirvish, S. S. (1983) The etiology of gastric cancer; intragastric nitrosamide formation and other theories. J. Natl. Cancer Inst. 71: 629–647

O'Neill, I. K., Castegnaro, M., Brouet, I., Povey, A. C. (1987) Magnetic semi-permeable polyethyleneimine microcapsules for monitoring of *N*-nitrosation in the gastrointestinal tract. Carcinogenesis 8: 1469–1474

Povey, A. C., Bartsch, H., Nixon, J. R., O'Neill, I. K. (1986) Trapping of chemical carcinogens with magnetic polyethyleneimine microcapsules: I. Microcapsule preparation and in-vitro reactivity of encapsulated nucleophiles. J. Pharm. Sci. 75: 831–836

Povey, A. C., Bartsch, H., O'Neill, I. K. (1987a) Magnetic polyethylenimine (PEI) microcapsules as retrievable traps for carcinogen electrophiles formed in the gastrointestinal tract. Cancer Letters 36: 45–53

Povey, A. C., Brouet, I., Bartsch, H., O'Neill, I. K. (1987b) Binding of benzo[*a*]pyrene metabolites in the rat intestinal lumen by magnetic polyethyleneimine microcapsules following an intragastric dose of [^{14}C]benzo[*a*]pyrene. Carcinogenesis 8: 825–831

Povey, A. C., Brouet, I., Nixon, J. R., O'Neill, I. K. (1987c) Trapping of chemical carcinogens with magnetic polyethyleneimine microcapsules: III. *In vivo* trapping of electrophiles from *N*-methyl-*N*-nitrosourea and recovery from feces. J. Pharm. Sci. 76: 201–207

Povey, A. C., Nixon, J. R., O'Neill, I. K. (1987d) Trapping of chemical carcinogens with magnetic polyethyleneimine microcapsules: II. Effect of membrane and reactant structures. Ibid. 76: 194–200

Poznansky, M. J., Juliano, R. L. (1984) Biological approaches to the controlled delivery of drugs: a critical review. Pharm. Rev. 36: 277–336

Sparks, R. E., Mason, N. S., Meier, P. M., Litt, M. H., Lindan, O. (1971) Removal of uremic waste metabolites from the intestinal tract by encapsulated carbon and oxidised starch. Trans Am. Soc. Artif. Int. Organs 17: 229–238

Ullberg, S. (1954) Acta Radiol. (Suppl.) 118: 1–110

Wilkins, T. D., Van Tassell, R. L. (1983) Production of intestinal mutagens, in: Hertges, D. J. (ed.) Human Intestinal Micro-flora in Health and Disease, Academic Press, New York, pp 265–288