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Radiolabelling of polymer microspheres for scintigraphic investigations by neutron activation. 4. A pharmacoscintigraphic study of colon-targeted Eudragit RS-sulphapyridine microspheres in human volunteers

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Summary

Dosage forms containing sulphapyridine have been administered to a group of five human subjects in a two-part combined pharmacokinetic and gamma scintigraphic study. In one portion of the study, subjects were administered enteric-coated capsules containing sulphapyridine powder and ^{99m}Tc-labelled ion-exchange resin. In the second portion of the study, the subjects were administered enteric-coated capsules containing ^{153}Sm-labelled sustained-release sulphapyridine-Eudragit RS microspheres. In both cases, the enteric-coated capsules were designed to deliver their contents into the caecum or ascending colon. Following capsule disintegration, the residence time of 50% of the radiolabelled microspheres in the ascending colon was 8.4 ± 2.0 h ($n = 5$), which did not differ significantly from the residence time of 50% of the radiolabelled ion-exchange resin (7.2 ± 1.9 h, $n = 5$). Pharmacokinetic analysis indicated a prolongation of drug release from the microsphere formulation. The bioavailability of sulphapyridine from the microspheres relative to the non-encapsulated drug powder was 41.3 ± 12.7% ($n = 4$). The reason for the reduction in drug absorption from the microsphere formulation was unclear although several theories are proposed.

Introduction

In recent publications, we have established that Eudragit RS microspheres may be radiolabelled, for gamma scintigraphic evaluation, by incorporation of samarium oxide (Sm_2O_3) followed by short exposure to neutron radiation (Watts et al., 1991a, 1993a). It was found that the extent of neutron exposure of the microspheres and the amount of incorporated target nuclide needed to be carefully balanced to maintain the physical properties of the microspheres, most importantly the drug release rate. For example, for Eudragit RS-sulphasalazine microspheres, high neutron exposure increased the drug release rate (Watts et al., 1993a), whereas for Eudragit RS-sulphapyridine microspheres, incorporating only 1.1% w/w Sm_2O_3 significantly suppressed the drug release rate (Watts et al., 1993b).

These studies have been undertaken as part of a research programme investigating the use of

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multiparticulate dosage forms for drug delivery into the colon. The ascending colon was of particular interest because of its inaccessibility by traditional means of colonic therapy such as enemas and suppositories. Although we have successfully prepared sustained-release Eudragit microspheres containing two colon-active agents, 5-aminosalicylic acid (5-ASA) (Watts et al., 1991b) and sulphasalazine (Watts et al., 1991a), these drugs were not ideal candidates as marker compounds to assess the microsphere in vivo performance. In the case of 5-ASA, poor colonic absorption would make pharmacokinetic analysis difficult. In the case of sulphasalazine, the molecule is selectively metabolised by colonic bacteria to generate 5-ASA and sulphapyridine (Klotz, 1985). Although sulphapyridine is well absorbed from the colon, with 70% bioavailability reported following sulphasalazine administration (Bieck, 1989), the quantity of sustained-release sulphasalazine microspheres required to generate measurable plasma levels would be prohibitive.

We therefore developed Eudragit RS microspheres containing sulphapyridine as a colon absorption marker (Watts et al., 1993b). In this paper, these microspheres have been radiolabelled with $^{153}Sm_2O_3$, delivered into the colon, and their passage followed using gamma scintigraphy. Colonic absorption of sulphapyridine released from the microspheres has been measured and compared to colon-targeted sulphapyridine powder in order to provide information on the pharmacokinetic performance of the sustained-release delivery system. Measurement of plasma sulphapyridine levels would also directly reflect the exposure of the colonic mucosa to drug, an important measure of the suitability of microsphere-based drug-delivery systems for providing drug for local topical action within the colon.

Materials and Methods

Materials

Eudragit RS100 (Dumas U.K., Tunbridge Wells, U.K.), sulphapyridine (gift from Rhône-Poulenc, Dagenham, U.K.), natural abundance samarium oxide (Sigma, Poole, U.K.), polysorbate (Tween) 20 (Sigma), sulphadimidine sodium (Rhône-Poulenc), Amberlite IRA410 resin (BDH, Poole, U.K.), sodium [^{99m}Tc]pertechnetate solution (eluted from Elumatic III generator, CIS International), methanol (HPLC grade) (Rhône-Poulenc), potassium dihydrogen phosphate (BDH) and sodium hydroxide (BDH) were obtained from the indicated sources.

Methods

Microsphere preparation Two 15 g microsphere batches were prepared. For each batch, 10 g of Eudragit RS was weighed into a glass bottle and dissolved in 100 ml of dichloromethane. When the polymer had dissolved, 225 mg of Sm_2O_3 was added to form a suspension. Dispersion of Sm_2O_3 was aided by sonication of the bottle contents for 10 min. Finally, 5 g of sulphapyridine powder was added to the bottle and dispersed by shaking. This mixture was immediately poured into 500 ml of 0.1% w/v aqueous Tween 20 solution and stirred at 250 rpm. Stirring was continued until the dichloromethane had evaporated, which took approx. 10 h. The hardened microspheres were then recovered by filtration, washed with 200 ml of distilled water, freeze-dried, and a 120–500 μm sieve fraction collected and weighed.

The collected sieve fractions from the two microsphere batches were placed into a single bottle and mixed together.

Microsphere characterisation Methods for microsphere characterisation are described in detail elsewhere (Watts et al., 1991a, 1993a). Briefly, microsphere drug content was determined spectrophotometrically and Sm_2O_3 content using X-ray fluorescence spectroscopy. The rate of drug release (into pH 6.8 phosphate buffer) was measured using the USP II method. Electron micrographs were obtained using low temperature (cryogenic) scanning electron microscopy.

Microsphere irradiation 3 g of the Eudragit RS-sulphapyridine microspheres were divided between two polypropylene tubes. The tubes containing the microspheres were irradiated at the Universities Research Reactor, Risley, U.K., for 15 min at a neutron flux of 10^{12} cm^{-2} s^{-1}.

The radioactivity of the neutron irradiated microspheres was measured using an isotope calibrator (Type 238, D.A.Pitman, Surrey, U.K.) set to detect ^{153}Sm.

Dosage form assembly Two types of dosage form were assembled for the two-part human investigation.

(i) Into a 000-sized hard gelatin capsule were weighed 500 mg of sulphapyridine powder and 100 mg of finely ground Amberlite IRA410 ion-exchange resin radiolabelled with ^{99m}Tc. The capsule was then dip-coated with an enteric polymer. The ion-exchange resin was included as a marker for capsule disintegration and as a control against which the transit rate of the radiolabelled microspheres could be compared.

(ii) Into a 000-sized hard gelatin capsule were weighed 130 mg of irradiated and 320 mg of non-irradiated Eudragit RS-sulphapyridine-Sm_2O_3 microspheres. The capsule was coated as described above. Only a portion of the microsphere dose was radiolabelled to ensure that the radioactivity was not too diffuse following capsule disintegration, which could result in poor quality scintigraphic images.

In vivo study design Five healthy male volunteers (age 23–29 years) were recruited for the study which received the prior approval of the University of Nottingham Medical School ethical committee and was conducted in accordance with the Declaration of Helsinki Guidelines for Ethics in Research. The study was carried out in two parts, separated by a period of 2 weeks.

On each occasion, subjects arrived at the study room at 7.30 a.m. having fasted from 10 p.m. the previous evening. A cannula was inserted into the forearm to enable blood samples to be withdrawn during the study day and markers containing a small quantity of neutron irradiated Sm_2O_3 powder were attached to the front and back over the right lobe of the liver to allow alignment during scintigraphic imaging. At approx. 8 a.m., subjects were asked to swallow, with a glass of water, either one capsule containing the sulphapyridine powder or three capsules containing the sulphapyridine microspheres. The former capsule contained 4 MBq of ^{99m}Tc whereas the latter three capsules contained a total of 1.5 MBq of ^{153}Sm. The quantity of sulphapyridine administered in each case was 500 mg.

Anterior and posterior scintigraphic images of 30 s duration were recorded at 30–60-min intervals throughout the day using an IGE Maxicamera II gamma camera (IGE, U.K.) fitted with a medium energy (300–100 keV) parallel collimator and stored on a Nuclear Diagnostics computer system (Nuclear Diagnostics, Gravesend, U.K.). Once the capsules had left the stomach, subjects received a light breakfast and thereafter a set lunch, coffee/tea and biscuits, and dinner. Blood samples were withdrawn at 0 h and approx. 2, 4, 6, 8, 11, and 13 h post-dose and immediately placed into heparinised tubes to prevent clotting. Tubes were centrifuged for 15 min at 2000 rpm and the plasma fraction removed and frozen at −20°C for later analysis. Subjects returned for scintigraphic imaging (120 s duration, microspheres only) and blood samples (by venipuncture) at 24 and 32 h post-dose. Images were not recorded at 24 and 32 h for the drug-powder leg of the study due to the short half-life of the ^{99m}Tc label (6 h).

Scintigraphic analysis Stored scintigraphic images were analysed to determine the distribution of activity between the ileo-caecal junction (ICJ), caecum/ascending colon, transverse colon and descending colon/rectum using methods described previously (Watts et al., 1992). The mean residence time of 50% of the administered radioactivity in the ascending colon was determined (MRT) and, for the microspheres only, the residual radioactivity remaining in the whole colon at 24 and 32 h.

Analysis of plasma samples Plasma samples were spiked with sulphadimidine sodium as the internal standard and analysed for sulphapyridine and its principal metabolite, acetylsulphapyridine. A mobile phase of pH 6.0 phosphate buffer containing 20% methanol was used at a flow rate of 1.2 ml/min (Knauer HPLC 64 pump, Berlin, Germany). Separation was performed using a Spherisorb ODS column (Hichrom, Reading, U.K.) and sulphapyridine and metabolites detected spectrophotometrically at 254 nm (Uvikon 720 LC detector, Kontron Instruments, Switzerland). Peak areas were calculated using a Spec-

tra-Physics 4400 integrator (Spectra-Physics, San Jose, U.S.A.).

Results and Discussion

Microsphere characterisation

The Eudragit RS-sulphapyridine microspheres contained 37.2% w/w sulphapyridine, representing an incorporation efficiency of 112%, and 1.12% w/w Sm_2O_3, representing an incorporation efficiency of 74.7%. In a previous paper it was noted that Sm_2O_3 appeared to increase the drug content of Eudragit RS-sulphapyridine microspheres (Watts et al., 1993b). By weight, 57% of the microspheres were in the size range 120–500 μm.

An SEM micrograph of a sample of non-irradiated microspheres is shown in Fig. 1. The microspheres appeared to be more mis-shapen than samples we had previously produced (Watts et al., 1993b). This could perhaps have been related to the batch size, which was more than 3-fold greater in this paper (15 g vs 4.5 g). The physical appearance of the microspheres was unchanged after irradiation.

The in vitro rate of sulphapyridine release from the microspheres was determined before and after irradiation and is shown in Fig. 2. The release rates were identical. After 12 and 24 h (not shown on graph), 75 and 93% of the encapsulated drug had been released, respectively.

The radioactivity generated by the 15 min irradiation was equivalent to 4.4 MBq/g of microspheres at the time of subject dosing (22 h after irradiation).

Transit rate

In the majority of instances, the coated gelatin capsules released their contents into the ileocae-

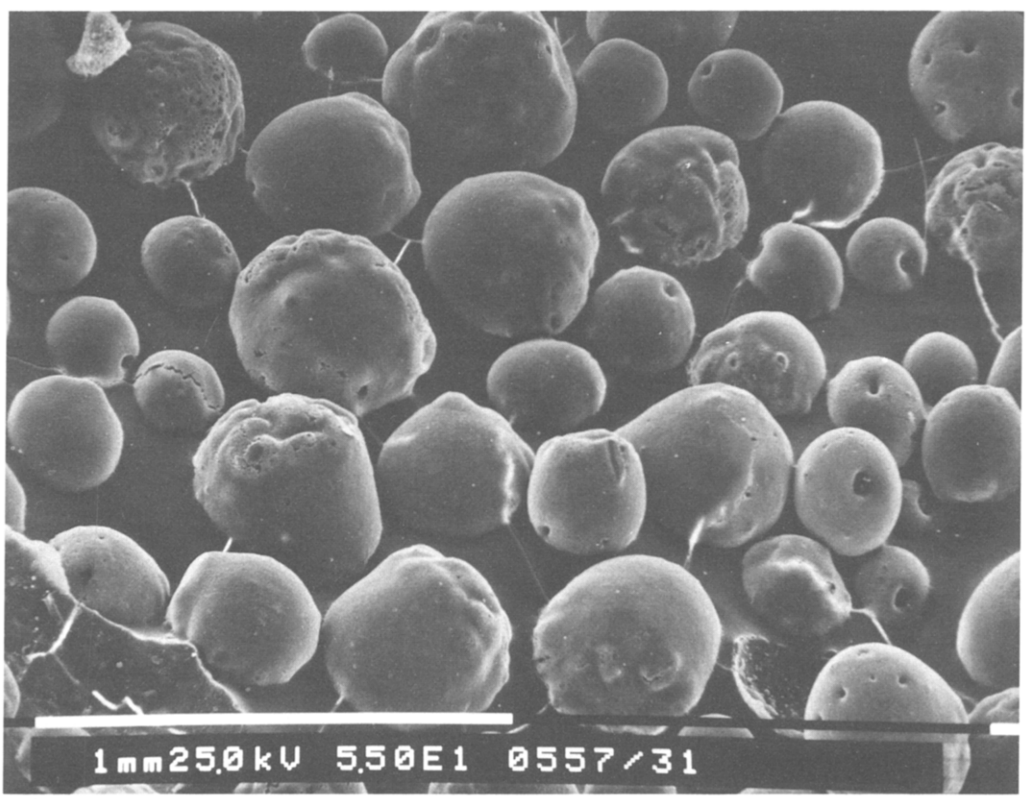

Fig. 1. Electron micrograph of Eudragit RS microspheres containing sulphapyridine and samarium oxide (magnification × 55).

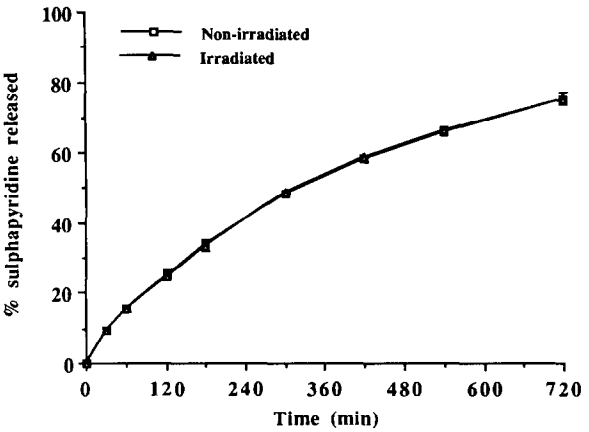

Fig. 2. Rate of sulphapyridine release from Eudragit RS microspheres before and after neutron irradiation.

cal junction or the caecum. However, in subjects 2 and 3, the capsules containing sulphapyridine powder disintegrated prematurely and the contents were released into the lower small intestine.

Table 1 presents the mean residence time (MRT) data for the two parts of the study. The mean MRTs of the 0.2 mm resin and the Eudragit microspheres in the ascending colon were 7.2 ± 1.9 and 8.4 ± 2.0 h, respectively. The difference between these values was not statistically significant.

The spread of the microspheres in the colon was wide and emphasized their suitability for delivering agents for topical treatment of an extensively diseased colon. This spreading might also serve to enhance the absorption of drugs for systemic action, by optimising contact between the carrier and the colonic mucosa.

TABLE 1

Mean residence time (MRT) of radiolabelled ion-exchange resin and Eudragit RS-sulphapyridine microspheres in the ascending colon

Subject	MRT (h)	
	Ion-exchange resin	Microspheres
1	5.2	7.3
2	5.4	8.3
3	7.3	11.7
4	8.0	8.3
5	9.9	6.6

TABLE 2

Percentage of microspheres residing in the ascending (AC), transverse (TC) and descending (DC) colon 24 and 32 h after administration

Subject	Percentage of administered microspheres remaining					
	At 24 h			At 32 h		
	AC	TC	DC	AC	TC	DC
1	22.6	36.4	29.4	16.2	28.2	28.3
2	1.7	5.7	7.6	3.4	0.0	1.1
3	19.5	25.7	35.3	16.1	15.3	0.0
4	8.1	1.6	16.8	7.4	1.6	0.3

Table 2 lists the proportion of the microspheres present in the ascending, transverse and descending colon for four of the subjects 24 and 32 h after administration (subject 5 withdrew from the 24 and 32 h imaging/blood sampling sessions). In subjects 1 and 3, in excess of 80% of the administered dose of microspheres was still resident in the colon after 24 h. In subjects 2 and 4, considerably less activity remained. There was no apparent correlation between MRT and whole colon transit. For example, the MRT in subjects 2 and 4 was no shorter than for subjects 1 and 3 despite the considerable difference in the proportion of activity remaining at 24 and 32 h. Presumably in subjects 2 and 4, loss of the radioactivity, by defaecation, had occurred between the end of imaging on the main study day and the 24 h image, and as a result the MRT was unaffected. Colonic mass movements, which may be associated with defaecation or meal intake, are an important determinant of colon residence. For example, a mass movement which resulted in approx. 80% of a dose of radiolabelled pellets moving from the ascending into the transverse and descending colon over a 15 min period has been reported (Proano et al., 1991). Therefore, ascending colon residence is not necessarily uniformly long, and in certain situations the opportunity for local drug action or absorption could be limited.

Pharmacokinetics

In the portion of the study involving administration of sulphapyridine powder, the parent drug

and its acetylated metabolite could be measured. However, when microspheres were administered, concentrations of the acetylated metabolite were in many instances too low to detect.

The mean plasma profiles of sulphapyridine following administration of the drug as powder and in microencapsulated form are presented in Fig. 3.

The area under the plasma concentration vs time curve (AUC) was calculated for the two formulations for each of the four subjects who completed the two portions of the study. By dividing the AUC for microencapsulated sulphapyridine by the AUC for sulphapyridine powder, the relative bioavailability of the microsphere formulation was estimated. The mean relative bioavailability of the microsphere formulation was 41.3 ± 12.7% (Table 3).

The relative bioavailability was lowest in subject 4. Although this may have been related to the low percentage of the administered microspheres resident in the colon at 24 h (Table 2), the amount remaining in subject 2 at 24 h was even lower, yet bioavailability was not affected to the same extent.

There are a number of possible explanations for the marked reduction in sulphapyridine bioavailability observed for the microsphere formulation.

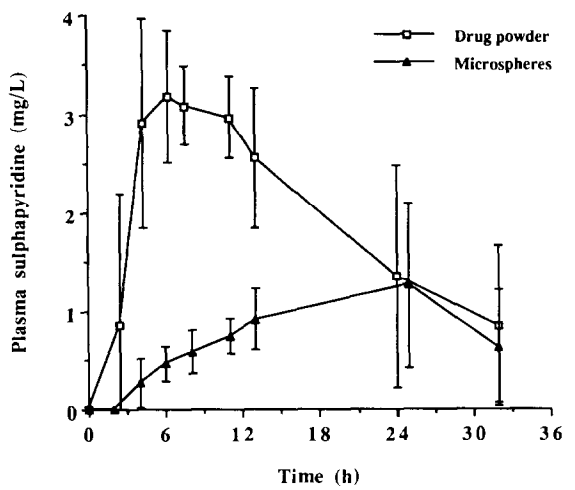

Fig. 3. Mean concentration-time profiles (±SD) for sulphapyridine administered intracolonically as a powder or in sustained-release Eudragit RS microspheres to four subjects.

TABLE 3

Bioavailability of sulphapyridine following administration as drug powder (p) or in Eudragit RS microspheres (m)

Subject	Relative bioavailability [AUC(m)/[AUC(p)]×100
1	50.0
2	50.5
3	41.3
4	23.4

The first might be in vitro/in vivo differences in drug release rate, for example, due to the degree of microsphere agitation or the viscosity of the colon contents.

Relatively little is known about the rate of drug absorption in the different regions of the colon. However, since the lumenal contents become more viscous in a distal direction, the efficiency of drug absorption might be expected to decrease correspondingly. This has been demonstrated for the antibiotic, ciprofloxacin, where absorption was lower in the left colon than in the right colon (Staib et al., 1989). Unfortunately, for the study described here, the relatively long-half life of sulphapyridine made assessment of declining drug absorption beyond the ascending colon difficult. Difficulty in assessment was also compounded by the fact that the drug carriers were of a highly dispersed nature and therefore drug may have been simultaneously released throughout a wide area of the colon.

It is also possible that a proportion of the sulphapyridine was sequestered by colonic bacteria. In common with other sulphonamide antibiotics, sulphapyridine inhibits bacterial folic acid production production by competing with gamma aminobutyric acid for the bacterial enzyme, dihydropteroate synthetase. Some of the sulphonamide is also incorporated into a folic acid analogue which disrupts various bacterial functions (Bowman and Rand, 1980). If sulphapyridine was captured by bacterial action, it would have the greatest effect on the bioavailability from the microsphere formulation: With sul-

phapyridine powder, lumenal concentrations would initially be very high, and could overwhelm the bacterial capture processes. However, with the sustained-release microspheres, lumenal drug concentrations would be very much lower, saturation of bacterial sequestration would not occur, and proportionally more of the dose of drug would be captured, resulting in reduced bioavailability.

The relatively long half-life of sulphapyridine also made the demonstration in vivo of microsphere sustained-release properties difficult, although the shape of the microsphere plasma profile (Fig. 3) did suggest an extension of release. To assess in a more quantitative manner the degree to which the microspheres demonstrated sustained-release properties, the drug mean residence time (dMRT) was calculated. The dMRT represents the average time that the drug administered resides in the body. It is calculated by dividing the AUC of the plasma concentration vs time curve by the area under the first moment of concentration vs time curve (AUMC) and subtracting the mean absorption time (Rowland and Tozer, 1989). For the purpose of demonstrating differences in dosage form performance, the dMRT was calculated by subtracting from [AUC/AUMC] the time at which drug absorption commenced, represented by the point of capsule disintegration on the scintigraphic images. The results of this analysis are presented in Table 4. The mean dMRT was significantly longer ($p < 0.05$) for the microsphere formulation (15.1 $\pm$ 3.1 h) than for the drug powder formulation (10.3 $\pm$ 1.7 h), implying a prolongation of drug release from this formulation.

TABLE 4

Mean residence time of sulphapyridine (dMRT) when administered as drug powder or in Eudragit RS microspheres

Subject	dMRT (h)		Difference [$m - p$] (h)
	Drug powder (p)	Microspheres (m)	
1	8.0	14.3	+6.3
2	12.8	17.9	+5.1
3	10.7	18.0	+7.3
4	9.8	10.4	+0.6

Conclusions

We have demonstrated that microspheres radiolabelled with $^{153}Sm_2O_3$ can be successfully visualised scintigraphically to provide information on their in vivo distribution in the human gastrointestinal tract.

The mean residence time of the microspheres in the ascending colon of approx. 8 h was generally comparable to a study investigating the colonic transit of model particulates and tablets (Watts et al., 1992).

There was good evidence to suggest a prolongation of drug release from the microspheres, both from drug mean residence time calculations and the extended appearance of the microsphere plasma-time profile.

The relative bioavailability of sulphapyridine absorbed from the sustained-release microspheres was less than 50% compared to non-encapsulated drug. There are potentially a number of factors which might explain the poor in vivo performance of the microspheres and without further work it is not possible to isolate any single one as being the most important.

In summary, this study has established methods for the in vivo biopharmaceutical evaluation of drug-polymer microspheres in the human colon. Further work is needed to assess their relative merits compared to other types of sustained-release dosage form and as a system for the local delivery of colon-active agents.

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