METHODS

Non-invasive method for delivery of tracer substances or small quantities of other materials to the colon

A Chacko, K F Szaz, J Howard, J H Cummings

Abstract
A miniature osmotic pump has been developed (Osmet) with ALZA, Palo Alto, USA, which can be swallowed, will pass through the stomach and small intestine and then deliver its contents (240 µl) over eight hours in the large bowel. In vitro studies showed the pumps started to discharge after four to five hours and emptied at a reasonably constant rate of 20-4 µl/h from 9-16 h (9-6%h). In vivo studies using gamma-scintigraphy in seven healthy subjects show that the pumps left the fasting stomach at 1-2 h (range 0-3) and arrived in the caecum by 6-4 h (range 5-9). Mean start-up time was 5-3 (0-2) h and the rate of discharge was 15-9 µl/h for pumps studied from 6-12 h and 17-2 µl/h for those studied from 10-20 h. This device is simple, safe and effective for the delivery of tracer substances to the caecum and colon without interfering with patients' normal lifestyle.

Delivering tracer substances, drugs or other materials directly into the lumen of the caecum and colon in man, whether for research or therapeutic purposes, poses considerable problems. Most substances given by mouth are absorbed from the small intestine and whilst some may be re-excreted in bile as conjugated forms oral dosing does not provide a reliable way of getting substantial amounts of many active compounds into the large bowel. Various enteric coatings have been devised to slow down the release in the small bowel. These often rely on changes in pH in the gut to determine onset of drug delivery. Unfortunately, there is no pH in the colon which is not encountered higher up in the gut so it is difficult to use this for reliable colonic dosing.

In the study of colonic metabolism a variety of techniques have been tried including intestinal intubation, breath hydrogen and methane measurements, rectal dialysis, extensive studies of faeces, ileostomy content, examination of necropsy material and in vitro work but these techniques either greatly disturb normal physiology or are so limited in scope that the overall role in digestive physiology and metabolism of the large bowel remains either unquantitated or unknown.

A system has therefore been developed which allows delivery of small quantities of known substances into the caecum using a miniature osmotic pump designed to pass through the stomach and small intestine before discharging its contents over an eight to 12 h period in the caecum and colon.

Methods

Osmet Pump (Fig 1)
This is a cylindrical device 25 mm×7 mm made from a styrene-butadiene copolymer and having a capacity of about 200 µl. An enteric coating designed to be insoluble in gastric juice but soluble at pH 7 covers the outside of the pump and the osmotic force is provided by a salt layer within the semipermeable copolymer exterior. The pump is filled with a syringe and blunt 25 gauge needle through the opening at one end,
after which a small plastic tube is inserted to regulate flow (flow moderator). Full details of the principle by which these pumps operate are available.\textsuperscript{14,15}

**IN VITRO STUDIES**

The rate of delivery from the pumps, the delay time to start up, and the duration of delivery were determined in vitro using eight pumps. The pumps were weighed, then filled with a solution containing 0.15 MBq \(^{14}C\)-PEG 4000, reweighed and then placed for one hour in a solution of HCl at pH 2 and for a further 23 h in simulated colonic fluid at pH 6.5 containing (mmol/l) K 70, Na 30, Ca 15, Mg 25, NH\(_4\) 10, acetate 98, propionate 32, butyrate 30, Cl 30, HCO\(_3\) 30, amylase 300 (BP U/ml), lipase 250, protease 14 (from Pancrex V powder 10 g/l), polyethylene glycol (PEG) 4000 5 g/l, and 12 ml/l of a solution containing a mixture of antibiotics (nalidixic acid 360 mg/l, vancomycin 48 mg/l, chloramphenicol 240 mg/l, neomycin 216 mg/l, penicillin 120 mg/l).

The studies were carried out in 50 ml conical flasks containing 20 ml fluid at 37°C and were shaken in an orbital incubator at 1000 rpm. All flasks were sampled at zero and 24 h and from four flasks samples were taken at hourly intervals from one to 14 h and from the other four flasks from 10--23 h.

Duplicate 100 μl samples of fluid from each flask were taken directly into glass liquid scintillation vials, 10 ml BDH Cocktail EX 'Scintron' added, the vial shaken then placed in a Philips scintillation counter PW 4540 at 4°C and left for two to three hours before being counted for two minutes. Standards of the original \(^{14}C\)-PEG were run and disintegrations per minute (dpm) calculated using external standardisation. Account was taken of the progressive decrease in volume of the solution in the conical flasks in calculating efflux of \(^{14}C\)-PEG from the pumps.

**IN VIVO STUDIES**

Seven healthy men aged 29--45 years took part in these studies. Pumps were filled with a solution containing 1 MBq \(^{99m}Tc\)-DTPA. In the first part of the study four subjects swallowed a pump on two occasions at 08:00 h after a 12 h fast. Together with the pump a 20 ml drink containing 3 MBq \(^{99m}Tc\) in water was given. The location of the pump in the gut and the amount of activity remaining within it was measured using gamma-scintigraphy hourly from 0--12 h and then at 24 h. In the second part of the study three subjects swallowed a pump on a total of four occasions at 10 pm (not fasting) and gamma-scintigraphy was performed hourly from 10--14 h then two hourly from 16--22 h.

During the study all subjects ate a controlled diet. In the first part there were two diets, either low fibre containing 9-0 kJ, 80 g protein, 64 g fat (P:S ratio 0:14), 330 g carbohydrate, and 13:5 g dietary fibre; or a high fibre diet containing 9-1 kJ, 80 g protein, 64 g fat (P:S ratio 0:29), 339 g carbohydrate, and 53 g fibre.\textsuperscript{16} Each of the four subjects were studied once whilst eating each diet. In the second part of the study all subjects ate the high fibre diet throughout. The subjects ate and drank normally during the study and pumps were recovered by faecal collection and residual activity measured in a gamma-counter.

**GAMMA-SCINTIGRAPHY**

Subjects were scanned whilst lying down using dual radionuclide imaging for 300 secs anteriorly and posteriorly, with an IGE maxicamera 400 AT. Data were recorded on a Nodelcroft V77 computer and stored before analysis. For \(^{11}In\) in a region of interest (ROI) was defined around the image of the pump on the visual display unit and the amount of activity in this area expressed as a fraction of total activity in the whole field of view. A geometric mean of anterior and posterior fractions was taken.\textsuperscript{17} The location of the pump was determined by inspection of the \(^{99m}Tc\) picture, which outlined the gut, with the \(^{99m}Tc\)-DTPA ROI superimposed.

The estimated effective dose equivalent to the whole body per administration was calculated to be 0.37 mSv for \(^{11}In\)-DTPA and 0.05 mSv for \(^{99m}Tc\) assuming an intestinal transit time of 48 h. The dose to the gut was 4.8 mSv for \(^{11}In\)-DTPA and 0.7 mSv for \(^{99m}Tc\).

The study was approved by the Ethical Committee of the Dunn Nutrition Unit on 18 November 1986 and the use of isotopes by the Administration of Radioactive Substances Advisory Committee (ARSAC) of the Department of Health and Social Security, Certificate No RPC-83-16/2.

**Results**

**IN VITRO STUDIES**

Average fill volume for the eight pumps was 241 μl (range 218--262). The four pumps monitored from 0--14 h started to deliver between four and five hours (an additional eight pumps used in preliminary studies also started to deliver at this time). Figure 2 shows that delivery rate gradually increased to a maximum over five hours and then from nine to 16 h was approximately constant at 20-4 μl/h during which time 67% of the dose was delivered. Pumps were emptied by 19--20 h and total recovery of the counts of \(^{14}C\)-PEG at 24 h was 102-6% (2-2% SEM). The proportion of the total dose from each pump emptying per hour was constant and is shown in Figure 3. From six to 17 h the pumps emptied at 8-2% per hour.

**IN VIVO STUDIES**

The pumps were swallowed without difficulty and led to no untoward effects. The position of the pumps in the gut was followed by gamma-scintigraphy with \(^{99m}Tc\) outlining the anatomy of the gut and the exact location of the pump definable by the \(^{11}In\)-DTPA image. Figure 4 shows a selection of scans from a single subject. In Figure 4a the \(^{99m}Tc\) lies almost entirely in the stomach (at 10 min) but the pump can be seen to move immediately into the duodenum. By one hour (4b) most of the small bowel is outlined by \(^{99m}Tc\) with the pump somewhere in the
middle. At seven hours (4c) the $^{99m}$Tc outlines the right colon with the pump visible in the caecum and still as a discrete circle indicating no significant discharge of contents. Subsequent films (4d–4f) show the pump traversing the colon with the bowel outlined by both $^{99m}$Tc and $^{111}$In-DTPA discharged from it. The onset of discharge from the pump was characterized by asymmetry in the shape of the $^{111}$In-DTPA image or the appearance of a trail of $^{111}$In-DTPA from the pump (Fig 4d) and by a reduction in the fraction of activity remaining in the region of interest. On average pumps had left the stomach by 1·2 h (Table) and arrived in the caecum by 6·4 h. The time spent by pumps in the small bowel, an average of 4·8 h (time from first observed in caecum–time left stomach) was fairly constant. Those pumps which took longest to reach the colon (A1 9 h; B1 8 h) also remained longest in the stomach (three hours each). Arrival in the caecum was apparently slower for the pumps given to non-fasting subjects at 10 pm. By 24 h the pumps were mostly in the left colon although two remained more proximally. The pump shown in Figure 4 (H in Table) reached the sigmoid colon by 12 h, which was quite rapid, and still contained 30% of the initial activity. It was passed the next day, after 23 h, and by then had only 1·1% of its original activity. Average transit time for all pumps (n = 12) was 31 h (range 19–55 h). Diet made no difference to the behaviour of the pumps except that overall transit time tended to be faster with the high fibre diet (transit time (h) low fibre diet 34·3 (6·3); high fibre 26·9 (2·4), t 0·97, n = 4 pairs).

Discharge from the pumps was first detected between five and seven hours, the mean start-up time being 5·3 (0·2) h. For pumps studied from 0–12 h a constant rate of discharge of 15·9 µl/h was seen from seven to 12 h and for pumps studied from 10–22 h the discharge rate was 17·2 µl/h from 10–20 h. No end point for emptying of the pumps could be determined because of difficulties in separating activity in the pump from that in the immediate vicinity towards the end of the study (18–24 h).

The amount of activity remaining in the pumps with time in vivo is shown in Figure 5. The rate of delivery was similar for pumps

<table>
<thead>
<tr>
<th>Study no</th>
<th>Left stomach by (h)</th>
<th>In caecum at (h)</th>
<th>Position at 24 h</th>
<th>Total transit time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>3</td>
<td>9</td>
<td>Transverse colon</td>
<td>53</td>
</tr>
<tr>
<td>A2</td>
<td>3</td>
<td>6</td>
<td>Splenic flexure</td>
<td>25</td>
</tr>
<tr>
<td>B1</td>
<td>3</td>
<td>8</td>
<td>Sigmoid</td>
<td>29</td>
</tr>
<tr>
<td>B2</td>
<td>3</td>
<td>6</td>
<td>Sigmoid</td>
<td>25</td>
</tr>
<tr>
<td>O5</td>
<td>3</td>
<td>6</td>
<td>Sigmoid</td>
<td>24</td>
</tr>
<tr>
<td>O6</td>
<td>1</td>
<td>5</td>
<td>Sigmoid</td>
<td>30</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>5</td>
<td>Sigmoid</td>
<td>25</td>
</tr>
<tr>
<td>H</td>
<td>0</td>
<td>6</td>
<td>Excreted</td>
<td>23</td>
</tr>
<tr>
<td>Mean A–H</td>
<td>1·2</td>
<td>6–4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: In vitro delivery rates of $^{14}$C-PEG from eight Osmet mini-pumps whilst being gently shaken from 0–1 h in HCl at pH 2 and from 1–24 h in stimulated colonic fluid at pH 6·5. All pumps sampled at 0 and 24 h. Pumps 1–4 sampled from 1–14 h and pumps 5–8 from 10–23 h. Mean (SEM).

Figure 3: % $^{14}$C-PEG remaining in eight Osmet mini-pumps studied in vitro. Conditions and sampling times as for Figure 2. Mean (SEM).

Figure 4: Gamma-camera pictures of one subject. Each view shows both anterior (upper views) and posterior (lower views) scans in the $^{99m}$Tc Technetium (left hand of pictures) and $^{111}$Indium (right hand) channels. The pump is best seen in the $^{111}$Indium channel. Its position has been outlined by computer and superimposed on the $^{99m}$Tc Technetium scan for the purposes of location. (a) 10 minutes after swallowing pump. The pump leaves the stomach and moves to the duodenum during the scan. Activity remaining in pump 97%. (b) One hour. Pump in upper small bowel which is outlined by $^{99m}$Tc. Activity remaining 97%. (c) Seven hours. Pump in caecum. $^{99m}$Tc outlines caecum and right colon. Activity remaining 86%. (d) Eight hours. Pump at hepatic flexure. Clear evidence of discharge of $^{111}$In from pump. Activity remaining 64%. (e) Nine hours. Pump in transverse colon. Large bowel outlined by both $^{99m}$Tc and $^{111}$In. Activity remaining 49%. (f) Twelve hours. Pump in sigmoid colon. Activity remaining 30%.
Non-invasive method for delivery of tracer substances or small quantities of other materials to the colon

by the pump is three to four hours, sufficient for it to reach the ileocaecal region in fasting subjects.

We undertook to compare the in vitro and in vivo performance of the pumps in order to establish their behaviour in man and also, given good comparability of the two systems, to allow future validation to be done mainly in vitro as the costs of in vivo validation using gamma-sciintigraphy are substantial.

In vitro validation is relatively straightforward although our initial attempts to do this with simulated colonic fluid and 4C glycine and 4C acetate were unsuccessful because of microbial degradation of these substances during the 24 h period at 37°C. We therefore chose to use a low concentration of relatively non-degradable 4C-PEG in addition to a cocktail of antibiotics in the test system. Overall recovery of 4C-PEG was close to 100%. An initial hour at pH 2 was chosen to simulate the time spent in the fasting stomach and pancreatic enzymes were added to the simulated colonic fluid since they are present in high concentrations in the large bowel as small bowel. In these conditions the pumps behaved uniformly well with a start up time of four to five hours.

The pumps were given to subjects fasting because in previous studies of the transit of different dosage forms through the gut by Davis et al5 pumps given with breakfast stayed in the stomach for 10–12 h. Whilst the coating on the present pumps might have prevented discharge of the contents during this time, it is preferable to aim to get the pumps to the colon as quickly as possible. Fasting subjects in the present study were allowed to eat normally as soon as the pumps had left the pyloroduodenal area. Pumps given at night to non-fasted subjects seemed to be delayed in their transit through the stomach and small bowel as only two out of four were at the caecum at 10 h. This delay in transit might be related to sleep as well as to giving the pumps with food.

By six to seven hours, the time by which the pumps were definitely in the caecum, a maximum of 10% of the dose had been delivered. The majority of the dose should therefore be released in the colon over the next 10-12 h. Although small, the fraction of the total dose delivered in the ileum needs to be taken into account in designing tracer studies. Substances released in the ileum will be absorbed and thus escape degradation by caecal flora. If the end-products of metabolism of material released by the pump are different by bacterial and absorbed routes then these may be distinguished in blood, urine etc. But if they are similar then the site of absorption will not be definable.

The amount of time the capsule spends in different regions of the gut may be determined, using either contrast media or 40In-DTPA, and may aid in quantifying potential small bowel delivery and the location of large bowel delivery also. From examination of the gamma-scan pictures it can be predicted that the greater part of the dose would be delivered proximal to the hepatic flexure. Of the eight pumps studied from 0–12 h, seven were still in the right colon or at the hepatic flexure at 12 h, whilst one (Fig 4) moved...
rapidly round the colon, passing the hepatic flexure at eight hours, at which time it had delivered 36% of its activity. Pumpings given at night reached the hepatic flexure at 12–23 h.

An alternative type of coating for these pumps that would target materials more specifically to the colon, would be one that was degraded by the microflora of the caecum. The time taken to degrade such a coating, however, together with the normal delay in start-up that is a characteristic of these pumps (one to two h) might mean that only a small proportion of the dose reached the right colon. Such a system might be ideal for left colonic delivery.

In vitro the pumps delivered at a maximal constant rate of 20·4 μl/h but in vivo the equivalent rates were 15·9 and 17·2 μl/h for the two sets of pumps. The reason for the apparently lower delivery rate in vivo relates to the problem of distinguishing activity remaining within the pump from that in the immediate vicinity. Whilst the pump is moving freely and the gut contents are relatively fluid, as in the right colon, material ejected is mixed with contents and moves away from the pump, enabling residual activity to be measured separately. In the left colon, however, when delivery is into a more solid matrix already containing isotope, and the amount of activity remaining in the pump is low, it becomes more difficult to separate the two. In vivo delivery rates are probably therefore an underestimate.

Alternatively if a subject passes a stool containing some isotope during the study then the overall activity of the field of view will be reduced giving an apparent increase in the fraction remaining in the pump.

One pump failed to empty and on recovery contained 95% of the original dose. Results from this pump are not reported but provided an unplanned for ‘control’ series of gamma-scintigraphy pictures. These showed that the fraction of total radioactivity represented by the region of interest remained constant throughout the study.

The osmotic pump therefore is a safe and effective device for the delivery of 200 μl into the caecum and colon of man without major disturbance to normal lifestyle. Because of the dimensions of the pump care must be taken, however, in its use in people with possible strictures of the gut. The operation of these pumps with different contents and coatings can be reasonably validated in vitro and provided they are given in the fasting state may be expected to deliver the majority of their contents in the caecum and right colon over an eight to 16 h period after ingestion. In preliminary studies we have used them to deliver 14N-glycine to the caecum to follow the incorporation of 14N into bacterial protein from its measurement in faeces and urine, and 14C-choleic acid in studies of caecal bile acid metabolism and diet by following 14C appearance in blood. They may also be used to target up to 200 mg of a drug to the caecum although their primary value long term is likely to be in research rather than therapeutics.

The authors are particularly grateful to Dr Philip Wright, Director of the Department of Nuclear Medicine at Addenbrooke’s Hospital for allowing use of his facilities for this study, and to Mr Mike Clay, Mr Ian Tabor, and Mrs Eileen Gribbon for their help with the gamma-scintigraphy. The coating for the pumps was developed by Dr Linda Goldman of ALZA and professor SS Davis, Nottingham University, gave valuable advice during the setting up of these studies. AC was in receipt of a grant from the Wellcome Trust. This work was presented at the annual meeting of the American Gastroenterology Association in 1987 and published in abstract form in Gastroenterology 1987; 92: A1340.

Non-invasive method for delivery of tracer substances or small quantities of other materials to the colon.
A Chacko, K F Szaz, J Howard and J H Cummings

Gut 1990 31: 106-110
doi: 10.1136/gut.31.1.106

Updated information and services can be found at:
http://gut.bmj.com/content/31/1/106

These include:
Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Stomach and duodenum (1689)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/