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Development and Validation of a Pig Model for Colon-specific Drug Delivery

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Abstract

The purpose of this investigation was to develop a pig model for colonic drug delivery and to validate the model by determining whether the physiology of the pig colon had been significantly altered after the surgical implantation of a gut cannula into the terminal ileum of the pig.

A fistula was created in the terminal ileum of the pig, and a cannula fitted for the purpose of directly administering drug formulations to a point just anterior to the ileocaeco-colonic valve of the gastrointestinal tract. The cephalic vein of the pig was also cannulated to enable continued blood sampling.

Sulphasalazine was used as the model drug for the validation study. In the intact colon, sulphasalazine is metabolized by the gut microflora to sulphapyridine which is then absorbed. Sulphasalazine was administered orally to non-fistulated and fistulated pigs and then ileally, via the gut cannula, to fistulated pigs. Absorption of sulphapyridine was monitored by HPLC analysis of plasma samples. There was no significant difference in the absorption obtained for the three groups. Thus it is demonstrated that the colon physiology had not been altered.

The colonic pig model is ideal for studying factors affecting the colonic absorption of drugs and as a means for developing drug delivery systems with improved absorption properties.

In the past decade there has been an increasing interest in peptides and proteins as therapeutics agents. However, a limiting factor in the acceptance and clinical use of such drugs is the need for delivery systems exploiting routes of delivery other than the parenteral injection. To date the oral route is still the most preferred and convenient method of drug administration. However with few exceptions, the bioavailability of peptides and proteins when administered by this route is low, generally less than 1% relative to intravenous administration. For example, oral formulations of desmopressin administered to healthy adults have been shown to have bioavailabilities in the order of 0.1 % (Fjellestad–Paulsen et al 1993). Low bioavailabilities have also been found in man for human calcitonin (Antonin et al 1992; Beglinger et al 1992). The poor oral absorption of such drugs is mainly due to the high degree of hydrophilicity and the large molecular weight of such molecules that make transport across the membrane difficult. Further, the molecules can be subjected to extensive enzymatic digestion in the lumen, the brush border region or in the cells lining the gastrointestinal tract. Although the surface area of the small intestine is large and therefore the opportunity for absorption should be better, the transit through the small intestine can be rapid with an average of 3 h from stomach to colon. Hence, the poor absorption of polypeptides when given orally could also be attributed to the short contact time between the molecule and the absorbing surface and the inability to produce high concentrations of drug and added excipients.

Recently work has focused on the colon as a potential delivery site for these poorly absorbed drug molecules (Davis 1990; Friend 1991; Ashford & Fell 1994). Although the surface area of the colon is much less than that of the small intestine and hence is not ideally suited for absorption, the environment is devoid of endogenous digestive enzymes other than those of microbial origin and the residence time in the colon can be as long as 10 to 24 h. Furthermore, little mixing takes place in the colon, which makes it possible to create local environments with optimal absorption conditions.

A number of approaches for the enhancement of absorption of polypeptides and other hydrophilic drugs from the colon has been explored, such as the use of absorption enhancers and stabilizing agents (Mrsny 1992). Most published studies on the effect of these systems have been performed in animal models such as the rat and the dog rather than man due to the lack of sufficient toxicity information on most of the compounds. Likewise, there is a need to investigate the oral and colonic absorption of novel drugs not yet tested in man. The rat is suitable for initial screening of absorption properties of drugs and effect of enhancers in the various parts of the gastrointestinal (GI) tract, but cannot be used to test prototype formulations and results cannot easily be extrapolated to man. The GI tract of the dog is not physiologically comparable with that of man and hence, certainly for the delivery (and absorption) of drugs in the colon, is an unsuitable model and often a poor predictor of the situation in man. This has created the need for an animal model in research which can be employed in rapid screening of potential absorption enhancers and novel drugs.

The pig is considered the most suitable animal model for oral and colon-specific drug delivery since it resembles the human situation better than any other non-primate mammalian species with respect to eating behaviour, anatomy and physiology of the GI tract (Fleming & Arce 1986; Rayner & Wenham 1986;

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Kararli 1995). Furthermore, Pond & Houpt (1978) pointed out that the pig is similar to humans in dental characteristics, renal morphology and physiology, eye structure and visual acuity, skin morphology and physiology, cardiovascular anatomy and physiology, and body weight. The digestive anatomy and physiology of the pig is similar to that of man with each section of the pig's GI tract being comparable anatomically with that of humans. Hence, the bacterial flora of the pig colon (Pond & Houpt 1978; Moran 1982; Miller & Ullrey 1987; Dressman & Yamada 1991) and the digestion characteristics of the small intestine are considered to be most similar to man (Rowan et al 1994). Pigs are also (as are most mammals) reported to possess an interdigestive, migrating myoelectric complex (MMC) which is known to be a critical factor in gastric emptying of indigestible solids in man (Ruckebusch & Bueno 1976). The small intestine transit time in the pig is very similar to that of man (Ruckebusch & Bueno 1976) whereas the gastric emptying has been shown to be slower (Hossain et al 1990; Aoyagi et al 1992). The pig is considered to be a superior model for colonic delivery, compared with smaller laboratory animals, since it is large enough for the administration of intact dosage forms intended for man. Hence, for these reasons it has been suggested by various authors that experimental data obtained in the pig model may be extrapolated to man (McClellan 1968; Hildebrand et al 1991). The pig has been used extensively as a model for human nutrition studies (Miller & Ullrey 1987; Moughan et al 1992; Darragh et al 1994; Rowan et al 1994) as well as a model in drug bioavailability studies (Koritz et al 1981; Bevill et al 1982; Aoyagi et al 1984; Hildebrand et al 1991; Larsen et al 1992).

The aim of the present work was to set up and validate a pig colon model in which to evaluate the colonic absorption of drugs by directly administering the formulation to a point just anterior to the ileo-caeco-colonic valve in the pig. Such dosing will avoid the problems of direct oral administration and variability in gastric emptying and small intestinal transit times. For this purpose a fistula was created in the terminal ileum of the pig and a cannula was fitted. The technique also avoided disturbing the anaerobic environment of the colon. The cephalic vein of the pig was also cannulated to enable blood sampling to monitor the systemic absorption of compounds from the colon.

Sulphasalazine was chosen as the model drug for the validation study. This compound consists of 5-amino salicylic acid linked to sulphapyridine by an azo-bond. When given orally, sulphasalazine reaches the colon mostly unchanged and is split bacterially by azo reduction into its parent compounds, 5-amino salicylic acid and sulphapyridine. The latter is extensively absorbed in the colon and can be easily detected in the plasma by HPLC (Das & Dubin 1976). The effective cleavage of sulphasalazine and the absorption of sulphapyridine depend on transit time and on an intact reductive colon (Klotz 1985). By administering sulphasalazine and monitoring the absorption of sulphapyridine in both fistulated and non-fistulated pigs, information could be gained on both the reductive capacity and the absorptive properties of the colon.

Materials and Methods

Chemicals

Sulphasalazine, sulphapyridine, sodium sulphamethazine and aspartame were obtained from Sigma Chemical Company, Poole, Dorset, UK. All other chemicals used were of analytical or HPLC grade.

Animals

Eleven male pigs (Large White cross, ranging in live weight from 27 to 43 kg) were housed in individual pens, located in an environmentally controlled metabolism room and kept under 24 h light, at 20°C. The pigs were fed twice a day with a standard grower/finisher diet and were given free access to water. Eight of the eleven pigs underwent surgery to cannulate the terminal ileum. These pigs were given a prophylactic course of antibiotics. The other three pigs underwent surgery to cannulate the cephalic vein.

Anaesthesia and gut surgery

The animals were fasted for 24 h before the operation. They were initially sedated with Stresnil, administered intramuscularly and were left undisturbed for 20 min after the injection. Anaesthesia was then induced with thiopentone injected intravenously and an endotracheal airway was inserted. Once intubated, general anaesthesia was maintained using a halothane-oxygen mixture. The pig was placed on its left side and the right lateral part of the abdomen was shaved, scrubbed with Savlon and swabbed with chlorhexidine. With full asceptic precautions a 9-cm incision was made in the midlateral part of the abdomen. The incision was made downwards at an angle of 45° to the horizontal, starting halfway down the side of the animal and from a point 5 cm behind the last rib. The underlying muscle layers and peritoneum were incised by blunt dissection exposing the visceral organs. The ileo-caecal junction was located and a loop of ileum exteriorized as close to the junction as possible. Where the ileo-caecal fold ended (5–10 cm from the ileo-caecal junction) the ileum and common mesentery were divided between vascular arcades so that the ileal blood supply was preserved. A purse-string suture was made, 4 cm in length using 3·5 (O) linen, on the antimesenteric side of the terminal ileum approximately 4–5 cm cranial to the ileo-caecal junction. A 3-cm incision was made within this suture and the T-piece cannula was inserted through this incision. The purse-string suture was used to secure the ileum around the base of the cannula stem. The stem of the cannula was exteriorized through a second smaller incision (2 cm) in the body wall. This incision was made 5 cm below and to the right of the original, larger abdominal incision. A retaining ring was used externally on the stem of the T-Piece to hold the cannula and terminal ileum in place against the side of the body wall. A central insert was then fitted inside the stem of the T-piece and a cap attached to hold this in place. The central insert contained a length of sialastic tubing which enabled solutions to be administered via a syringe into the terminal ileum. The larger abdominal incision was closed (using 4/0 vicryl) in four stages so that each muscle layer was individualy sutured. Following surgery, each pig was given a course of in-feed antibiotics, 75 mg kg^{-1} Tribrissen powder per day and a course of antibiotic injection, Tribrissen 48% injection intramuscularly. The pigs were then given at least a two-week recovery period before undergoing surgery to cannulate the cephalic vein.

Cephalic vein cannulation

After fasting for 12 h the animals were anaesthetized in the same way as for gut surgery. The pig was then placed on its left

side and the top of the right front leg and the area cranial to the right shoulder blade on the neck of the pig was shaved, scrubbed with Savlon and swabbed with chlorhexidine. The cephalic vein was surgically exposed, just above the junction of the right leg to the body, by making a 2-cm skin incision. The distal end of the vein was tied and a 5 French cephalic vein catheter was put in place. The tip was passed cranially until an approximate 30-cm length of catheter tubing had been inserted. The catheter was secured in the vein with two non-occluding sutures. The free end of the catheter was tunnelled subcutaneously and exteriorized at the back of the neck. An infusion of 50 int. units mL^{-1} heparinized saline was given. After surgery each pig was given Terramycin L.A., intramuscularly to counteract post-operative infection. Two days after vein catheterization the pigs were ready for use in the validation study. During the course of the study they were housed in metabolism crates.

Experimental procedures

For the study the pigs were divided into three groups of three or four pigs. Group 1, comprising three non-fistulated pigs, was administered sulphasalazine (2 g) orally. Group 2, comprising four fistulated pigs was administered sulphasalazine (2 g) orally. Group 3, comprising four fistulated pigs was administered sulphasalazine (0·5 g) as a suspension, through the ileal fistula into the terminal ileum.

Dose administration

For Groups 1 and 2 the pigs were fasted overnight but were allowed free access to water. The dose was administered to each pig by mixing 2 g sulphasalazine with a minimum quantity of pig feed (250 g). To mask the bitter taste of the drug, aspartame (20 mg) was added as a sweetener. Water (100 mL) was mixed with the feed to give the mixture the consistency of a thick paste. The remainder of the feed (750 g) was given 1 h after finishing the dose. For Group 3, the pigs were fed as normal and the dose of sulphasalazine (0·5 g) was administered as a suspension through the ileal fistula, via the tubing, while the pigs were feeding. The dose of sulphasalazine was suspended in 10 mL buffer (60 mM $NaHCO_3$, 8O mM NaCl) which was selected to mimic the ionic composition of the GI fluid. A 20-mL syringe was used to flush the 10 mL dose suspension through the length of tubing, in the gut cannula, into the terminal ileum. The suspension was flushed through completely with a further 10 mL buffer. The ileal dose of sulphasalazine was reduced from the oral dose of 2 g to 0·5 g to avoid possible toxicological effects since sulphasalazine was administered directly to the colon. Within the dose range used the kinetics of absorption was considered linear.

Blood sampling

Blood samples (4 mL) were taken from the cephalic cannula and collected into heparinized tubes. The patency of the catheter was maintained by flushing with 4 mL heparinized saline (50 int. units mL^{-1}) after each blood sample was taken. Hence the first few millilitres of blood was discarded each time a blood sample was taken. For all the groups a control blood sample was taken just before dosing. For Group 1 and Group 2, blood was collected at 3, 4·5, 6, 9, 12, 24, 30, 36, 48, 72 and 96 h after dose administration. For Group 3, blood was collected at 1, 2, 3, 4, 5, 6, 9, 12, 24, 36, 48, 72 and 96 h after dose administration. The blood was kept on ice until plasma separation. Plasma was separated by centrifugation at 3000 rev min^{-1} and 4°C. The plasma samples were transferred into polypropylene tubes and stored at −20°C until analysed by HPLC.

It should be noted that most scrupulous care must be taken with cleanliness of all procedures not only during operational procedures but also during dosing and especially blood sampling to avoid sepsis.

HPLC analysis

The concentrations of sulphapyridine in the plasma samples were determined by HPLC. The method was based on the method of Astbury & Dixon (1987) as modified by Watts et al (1994). Plasma samples (0·5 mL, in duplicate) were diluted, and precipitated, with methanol (0·5 mL) containing a constant concentration of the internal standard, sulphamethazine, for the assay. The samples were centrifuged and 20-μL volumes of the supernatant were analysed by HPLC using isocratic elution. A mobile phase of pH 6·0, 0·05 M phosphate buffer containing 10% methanol was used at a flow rate of 1·2 mL min^{-1}. Separation was performed using a reverse-phase Spherisorb ODS column. Sulphapyridine and sulphamethazine were detected by UV absorbance at 285 nm and the ratio of peak areas (sulphapyridine : sulphamethazine) was calculated for each sample. The concentrations of sulphapyridine in the experimental samples were then determined from the calibration curve.

Calibration samples were prepared in control (pre-dose) plasma containing a range of concentrations of sulphapyridine corresponding to 1–50 μg mL^{-1}. The same constant concentration of sulphamethazine as in the test samples was added. A calibration curve was drawn of peak area ratio (sulphapyridine : sulphamethazine) against sulphapyridine concentration.

The detection limit for the assay was 2 μg mL^{-1}. Therefore for the experimental plasma samples, sulphapyridine concentrations of less than 2 μg mL^{-1} were recorded as < 2 μg mL^{-1} and were regarded as 0 μg mL^{-1}, for the purpose of further calculations.

Calculations

For each animal a graph was drawn of plasma sulphapyridine concentrations against time. The area under the curve (AUC) was calculated by a trapezoidal method using a Minim 1·9c computer program. These area values represent a measure of the relative bioavailability of sulphasalazine. For the oral administration of sulphasalazine the dose was fixed at 2 g sulphasalazine per pig and at 0·5 g sulphasalazine per pig for ileal dosing. To obtain a direct comparison, the AUC for each pig had to be corrected for the dose received per kg.

$$\text{Corrected AUC dose (kg h mL}^{-1}) = \frac{AUC\ (\mu\text{g mL}^{-1}\text{h})}{\text{Dose per kg } (\mu\text{g kg}^{-1})}$$

Comparisons between the corrected AUC values of the three groups were performed statistically with an unpaired Student's *t*-test. A level of $P < 0{\cdot}05$ was considered significant.

Results

Fig. 1 shows the mean plasma concentrations of sulphapyridine against time after the oral administration of sulphasalazine to

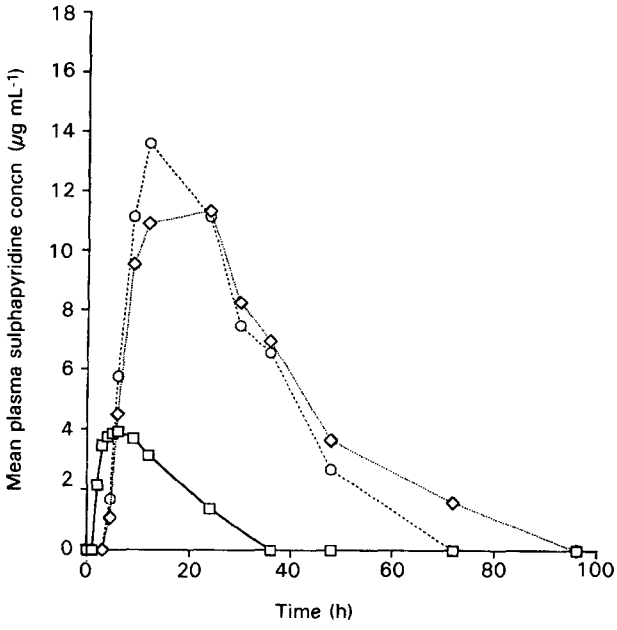

Fig. 1. Mean plasma concentrations of sulphapyridine following the (◇) oral administration of 2 g sulphasalazine to non-fistulated pigs, the (○) oral administration of 2 g sulphasalazine to fistulated pigs, the (□) ileal administration of sulphasalazine to fistulated pigs.

non-fistulated pigs (Group 1), fistulated pigs (Group 2) and the ileal dose of sulphasalazine to fistulated pigs (Group 3), respectively. For Groups 1 and 2 the maximum plasma sulphapyridine concentrations were reached between 12 and 24 h after dose administration whereas for Group 3 the maximum plasma sulphapyridine concentrations were reached at approximately 6 h after dose administration. The AUC values and corrected AUC values are shown in Table 1. No significant difference between the values obtained for the three groups was found.

Discussion

The similarity in corrected AUC values between the two orally dosed groups demonstrated that normal bacterial metabolism of sulphasalazine to sulphapyridine, and subsequent absorption of the sulphapyridine produced, was occurring in the surgically modified pigs, thus indicating that the surgery has not significantly altered the colonic environment. The similarity in the corrected AUC values between the two orally-dosed groups and the ileally-dosed group indicates that the process of opening the ileal fistula and administering the dose directly to the terminal ileum, does not significantly affect the reducing and absorptive properties of the colon. The results obtained from Groups 1 and 2 compare well with a similar study performed in six human subjects (Bieck 1989). In this study, an oral dose of 2·5 g sulphasalazine was administered and the plasma concentration–time curve obtained showed that there was a continuous absorption of sulphapyridine from the colon between 5 and 24 h after oral intake of sulphasalazine. A peak of 18 $\mu g\ mL^{-1}$ sulphapyridine was obtained at 25 h, after this the concentration decreased but the compound was still detected 85 h after drug administration. Differences in time after drug administration for peak plasma sulphapyridine concentrations are possibly linked to variation in transit times between individual pigs. A similar study in man looked at the variability of orocaecal transit time using the sulphasalazine/sulphapyridine method (Gramatté & Terhaag 1991). In this study sulphasalazine was administered as an oral bolus and the subsequent rise of sulphapyridine in the plasma was used to determine the orocaecal transit time. The study showed marked differences in both intra-subject and inter-subject orocaecal transit times. These differences could be due to different amounts of sulphasalazine reaching the large intestine, variable quantities of sulphapyridine produced by the resident bacteria or great variation in the rate and amount of absorption of sulphapyridine from the large intestine (Gramatté & Terhaag 1991; Dhote et al 1995). Large individual variations in small intestinal transit time were reported to be correlated with individual variations in gastric emptying when measured by the lactulose breath hydrogen test (Caride et al 1984).

A similar surgically modified pig model has been used previously by Elias et al (1992) to study the portal and systemic absorption of insulin after direct colonic administration. However, in this work the pigs were kept under general anaesthesia during the whole of the experiment and killed at the end of the experiment. The surgically modified pigs described here, once recovered after the surgery, can be used experimentally in the conscious state. Further, the same animal can be used for a large number of experiments thus minimizing the number of animals and variations per experiment by allowing for cross-over studies in the same group of animals. The life-time of the model is limited only by the growth of the animal which makes it too large to handle once the weight of approximately 90 kg is reached.

Due to problems with post-catheterization infections in some pigs in connection with blood sampling, in later studies a different technique for blood sampling was introduced involving a subcutaneous sampling port located on the right side of the neck of the pig and connected as previously to the cephalic vein. A scrupulous skin-cleaning procedure was also implemented prior to puncture of skin for access to the sampling port to avoid any sepsis occurring.

The pig colon model is currently being used to study the factors affecting the colonic absorption of peptides and proteins and the possibility of developing delivery systems for such drugs with improved absorption using concepts of the creation of local microenvironments within the colon with optimal absorption conditions. For this purpose the developed colonic pig model is ideal in that the solid formulations can be delivered directly to the terminal ileum through the ileal fistula thereby avoiding problems with oral dosing of large unit dosage forms.

Table 1. Experimental group characteristics and pharmacokinetic data.

Group	Pig weight (kg)	C_{max} ($\mu g\ mL^{-1}$)	T_{max} (h)	AUC ($\mu g\ mL^{-1}$ h)	Corrected AUC ($\times 10^{-4}$ kg mL^{-1} h)
1	34–37	11·3	24	327·4 ± 57·8	56·7 ± 14·5
2	27–31	13·6	12	362·4 ± 54·3	55·0 ± 11·1
3	40–43	3·9	6	58·2 ± 24·9	49·0 ± 21·6

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