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Colonic delivery of dexamethasone: a pharmacoscintigraphic evaluation

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SUMMARY

Background: Colonic delivery of corticosteroids may reduce the side-effects commonly associated with their use. Therefore, we tested the ability of the naturally occurring polysaccharide guar gum to deliver a corticosteroid, dexamethasone, to the colon using pharmacoscintigraphy. Guar gum is metabolized in the colon by resident bacterial enzymes to trigger drug release.
Materials: Each subject (eight per group, parallel study design) was administered one of four dexamethasone (9 mg) tablet formulations, radiolabelled with ^{153}Sm using neutron activation, under fasted conditions. One formulation was designed to release drug rapidly following ingestion while the other three formulations were designed to delay release of dexamethasone to varying degrees. Progression of the formulations down the gastrointestinal tract was followed by gamma scintigraphy. Serum concentrations were measured over time to relate disintegration profiles of the tablets with pharmacokinetic observations.
Results: The immediate release formulation disintegrated in the stomach, on average, within 20 min of dosing. One of the three delayed release preparations (CD_1) began to disintegrate in the small intestine 1.7 ± 1.0 h after dosing. The second and third delayed release preparations (CD_2 and CD_3) did not begin to disintegrate until 5.8 ± 2.3 and 3.6 ± 1.6 h after dosing, respectively. All three colonic delivery preparations completely disintegrated in the colon ranging from 7.8 ± 2.7 h (CD_1) to 12.4 ± 3.2 h (CD_2) following oral administration. Pharmacoscintigraphic data indicated that 72–82% of the dexamethasone was delivered into the colon although not all the dexamethasone delivered into the colon was absorbed.
Conclusions: Simple guar gum formulations are capable of delivering the corticosteroid dexamethasone to the colon of normal subjects. Locally delivered corticosteroids may be useful in the treatment of ulcerative colitis and Crohn's disease. Pharmacoscintigraphic evaluation is a useful method to discriminate between the *in vivo* behaviour of colonic delivery systems.

INTRODUCTION

Delivery of drugs to the colon is useful in the treatment of several colonic diseases (ulcerative colitis and Crohn's disease). Corticosteroids have traditionally formed the basis of treating inflammatory bowel disease. However, chronic treatment of inflammatory bowel disease with steroids, while often effective, is plagued by a number of serious side-effects (e.g. acne, moonface, striae, hypertension, peptic ulcer, impaired glucose tolerance and mood disturbances). Long-term treatment (greater than 6 months) can lead to osteoporosis, cataracts, necrosis and fracture of head and femur, and overt diabetes mellitus. If these undesired side-effects could be overcome or markedly reduced in both subchronic and chronic dosing regimes, corticosteroids would have the potential

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of being ideal therapeutic treatments of inflammatory bowel disease.[1]

One approach to improving the use of corticosteroids to treat inflammatory bowel disease is through site-specific drug delivery. By delivering more drug to the inflamed tissues, it is possible to substantially increase the local tissue concentrations. Kinetically, local delivery is assessed using a term called drug delivery index.[2] By increasing the drug delivery index, it is possible to deliver efficacious amounts of drug from significantly smaller doses. As a result of the lower dose, side-effects should be reduced relative to traditional approaches to drug administration. This concept has been successfully demonstrated in a number of animal studies involving oral administration of colonic delivery systems for corticosteroids[3,4] and in humans with delivery via enemas.[5]

The general approaches to delivering drugs to the colon via the oral route include: (1) enteric coating designed to release drug in the more alkaline environment of the gastrointestinal tract;[6] (2) bioerodible coatings and matrices;[7] (3) prodrugs;[8] (4) timed—release systems;[9] and (5) sustained release systems that release drug as they transit through the small and large intestines.[10] An alternative to oral administration is rectal dosing (suppositories, enemas).[11]

A potential matrix material for colonic drug delivery is guar gum. Owing to its high viscosity this polysaccharide may carry certain drugs to the large intestine without appreciable release in the stomach or small intestine. Once in the large intestine, the guar gum matrix will be degraded by specific enzymes produced by the gut microflora (i.e. α-galactosidases and β-mannanases) to initiate drug release.[12]

In the early stages of product development of novel colonic delivery systems, considerable time can be lost in establishing the likely potential of any given research strategy because of a lack of suitable *in vitro* or animal models. Pilot pharmacokinetic studies in small groups of healthy subjects may provide unsuitable end-points for the assessment of colonic targeting preparations.[13] However, pharmacoscintigraphic evaluation provides detailed information on the *in vivo* performance of novel oral formulations and can be used to focus the product development process.[14]

The purpose of this study was, therefore, to determine the *in vivo* performance of selected formulations for the guar gum colonic drug delivery research programme. The investigation was a pharmacoscintigraphic evaluation of guar gum delivery formulations spanning a range of release profiles. Disintegration and gastrointestinal transit data determined scintigraphically were correlated with the absorption profiles of dexamethasone.

SUBJECTS AND METHODS

Subjects

A total of 32 healthy volunteers (18 male and 14 female) were enrolled in the study. Each subject underwent a medical examination both prior to and following completion of the study, during which blood samples for haematology and clinical chemistry were taken, and a urine sample was provided for urinalysis. The protocol of the study was approved by an IRB (Quorn Research Review Committee, Leicestershire, UK). Approval to administer radiolabelled preparations to healthy volunteers was obtained from the Department of Health, London. Prior to recruitment, the nature of the study was explained both verbally and in writing to the volunteers, and each volunteer provided written consent.

Experimental procedures

This investigation was a double-blind, parallel group design in which blocks of eight healthy subjects received one of four different treatment regimens. A total of 32 healthy male or non-pregnant, non-breast feeding female subjects participated; subjects were randomized to one of the four different treatment formulations so that each subject received only a single dose. The four treatment formulations were designed to release dexamethasone to varying extents in the gastrointestinal tract. The first formulation was an immediate release (IR) tablet while the remaining three tablet formulations were designed to release drug primarily in the colon and are designated as CD_1, CD_2 and CD_3. Dexamethasone (U.S.P., micronized) was obtained from the Upjohn Co. (Kalamazoo, Michigan, USA) and the formulations were manufactured by Penn Pharmaceuticals (Tredegar, UK). These tablets differed with respect to grade (particle size) of guar gum and other excipients used to modulate drug release in the gastrointestinal tract. The tablets weighed approximately 333 mg and contained 2.7% dexamethasone (i.e. 9 mg of dexamethasone per tablet) and 2 mg of samarium oxide. The tablets were irradiated for 6 min in a neutron flux of 10^{12} n/s.cm^2, and *in vitro* dissolution testing demonstrated that neither the addition of the samarium oxide nor the neutron activation process

affected the performance of the dosage forms or the stability of the drug.

Anterior and lateral anatomical markers containing 0.1 MBq $^{99}Tc^{m}$ were taped to the skin over the right lobe of the liver. On a single study day, each subject received a single formulation radiolabelled with ^{153}Sm and containing 9 mg dexamethasone at approximately 08.00 h with 240 mL of water. Anterior scintigraphic images were recorded at frequent intervals for up to 16 h, using a gamma camera (General Electric Maxicamera) with a 40 cm field of view and fitted with a low-energy parallel hole collimator. Images were recorded at approximately 10-min intervals up to 12 h post-dose and then at approximately 30-min intervals until 16 h post-dose. Return visits were made to the clinical unit at 24 and 36 h post-dose to allow the acquisition of further images. The volunteers remained moderately active during the study period and all images were acquired with the subjects standing in front of the gamma camera. The images were recorded using a Bartec computer system and were stored on optical disk for subsequent analysis.

Transit and tablet disintegration information related to the following parameters were obtained: (a) gastric emptying time; (b) colon arrival time; (c) small intestinal transit time; (d) anatomical location and time of initial tablet disintegration; and (e) anatomical location and time of complete tablet disintegration.

A standard light lunch, dinner and supper were provided at 4, 9 and 14 h post-dose, respectively. Each subject drank 200 mL of water at 2 h post-dose and fluids were allowed *ad libitum* after lunch. At the end of study day 1, subjects were instructed to fast until returning to the clinical unit the following morning. Food was only allowed *ad libitum* after the 24-h image and blood sample.

Venous blood samples were withdrawn via an intravenous cannula or by venipuncture on the following time schedule: 0 (pre-dose), 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 24.0 and 36.0 h post-dose. The samples were left at room temperature for approximately 30 min until a clot was formed. The samples were then centrifuged at approximately 3000 r.p.m. (or 1800 ***g***) for 7 min at 4 °C. The resulting serum fraction was split into two aliquots by pipetting into two pre-labelled polypropylene screw-cap tubes. Samples were flash frozen and then stored immediately at −20 °C for radioimmunassay (RIA) of dexamethasone concentrations. Plasma cortisol levels were also measured by RIA as there was some cross-reactivity with cortisol in the dexamethasone RIA technique.

Analysis of dexamethasone and cortisol

Dexamethasone was measured in serum using a validated radioimmunassay method by Phoenix International Life Sciences (St. Laurent, Quebec, Canada). Dexamethasone (Reference Standard) for assay development was obtained from Sigma Chemical Co. (St Louis, Missouri, USA). Sheep anti-dexamethasone antisera was purchased from Guildhay Ltd. (Guildford, Surrey, UK) and [1,2,4-^{3}H]-dexamethasone was purchased from Amersham Canada Ltd (Oakville, Ontario, Canada). Rabbit anti-sheep whole serum was purchased from Sigma Chemical Co. and human serum was purchased from Scantibodies Laboratory (Santee, California, USA). Cortisol reference standard was purchased from American Chemicals Ltd (Montreal, Quebec, Canada). Coat-A-Count cortisol RIA kits were purchased from Diagnostics Product Corporation, DPC (Los Angeles, California, USA) for cross-reactivity evaluation of anti-dexamethasone antibody with cortisol.

The method involved allowing the tritiated dexamethasone and non-radioactive dexamethasone to react with the sheep anti-dexamethasone antiserum under suitable incubation conditions and for an adequate period. Separation of free dexamethasone from the antibody-bound dexamethasone was achieved by adding the second antibody (anti-sheep whole serum) plus polyethylene glycol (PEG 8000). The antibody-free fraction was measured by liquid scintillation counting. The concentration of dexamethasone in the unknown samples is directly proportional to the interpolated counts of the free fraction, obtained from a standard curve containing known amounts of dexamethasone. The range of quantitation of dexamethasone was 202–2504 pg/mL with a lower limit of quantitation (LLOQ) of 2020 pg/mL and lower limit of detection (LLOD) of 49.5 pg/mL. The range of quantitation of cortisol was 10.0–500 ng/mL with a LLOQ of 10 ng/mL and a LLOD of 1.2 ng/mL.

Pharmacokinetics

A variety of pharmacokinetic parameters (e.g. area under the concentration-time curve (*AUC*), mean residence time (MRT) and mean absorption time (MAT) were measured using standard techniques. The amount of drug absorbed over time was estimated using a Wager–Nelson calculation[15] and reference pharmacokinetic parameters.[16] In order to assess the impact of observed non-zero pre-dose concentration values due to cross-reactivity

with serum cortisol on the AUC_{inf}, the baseline value was subtracted from all time points and the mean corrected AUC_{inf} was compared to the mean uncorrected AUC_{inf}. The impact of baseline adjustments on the mean AUC_{inf} of the uncorrected *vs*. the corrected concentration values for all four formulations was minimal, since this difference ranged from 1 to 3%. Therefore, non-corrected data are presented.

Statistical analysis

Analyses of variance were performed on the untransformed pharmacokinetic parameters listed above, with the exception of the ratio AUC_{0-t} to AUC_{inf}, elimination rate constant following intravenous administration ($k_{el(iv)}$), MRT and MAT. The analysis of variance model included drug formulation as a factor. A 5% level of significance was used. Each analysis of variance included calculation of least-squares means, adjusted differences between formulation means and the standard error associated with these differences. The above statistical analyses were done using the SAS GLM procedure.

RESULTS

The tablets were observed to be present in the stomach in all 32 individuals in the first scintigraphic image following dosing (i.e. approximately 1 min post-dose). They did not adhere to the oesophageal wall nor subsequently obstruct the gut.

It was noted that a small amount of radioactive marker 'leached' from each of the four formulations shortly after administration of the preparations. Initially, this marker was observed to disperse throughout the gastrointestinal tract; however, it often could not be detected in subsequent images. This material is thought to result from progressive erosion of the surface of the tablets due to the continual peristaltic action of the gut. For this reason, initial disintegration was recorded as the midpoint between the two images after which dispersed radioactive marker was observed in consecutive images.

Table 1. Transit profiles of the four dexamethasone delivery systems evaluated

Formulation	Gastric emptying (h post-dose)	Colon arrival (h post-dose)	Small intestinal transit (h)
IR	NA*	NA	NA
CD_1	0.9 ± 0.7†	5.2 ± 1.5	4.3 ± 1.6
CD_2	0.6 ± 0.4	4.2 ± 0.6	3.6 ± 0.6
CD_3	0.9 ± 0.7	4.9 ± 1.2	4.1 ± 1.4

* NA = not applicable as there was no discrete transit event due to prior disintegration of the tablets.
† Data are means ± s.d. ($n = 8$).

Mean transit data (gastric emptying (GE), colonic arrival time and small intestinal transit time (SITT)) for all four formulations are summarized in Table 1. Formulation IR disintegrated before gastric emptying could be observed while the remaining three formulations remained intact until reaching the distal small intestine or colon. Table 2 summarizes the mean disintegration times of all four formulations tested.

Initial disintegration of formulation IR occurred in the stomach at 10 ± 17 min (range 1–48 min) post-dose and tablets completely disintegrated shortly after administration in six of the eight subjects (25 min ± 5.16 h; range 1 min–13.8 h ($n = 7$) post-dose). The result was a sharp rise in the serum dexamethasone concentration within the first hour after dosing (see Figure 1).

Formulation CD_1 initially disintegrated in the small intestine (1.7 ± 1.0 h; range 0.7–3.8 h; see Table 2). Because initial disintegration occurred in the more proximal portion of the intestine, some drug was absorbed before reaching the colon (see Figure 1). Colon arrival occurred 5.2 ± 1.5 h (range 3.9–7.6 h) post-dose. Complete tablet disintegration occurred in the colon at 7.9 ± 2.7 h (range 5.1 ± 12.8 h; $n = 8$) post-dose. Total dexamethasone absorption was complete by 16 h post-dose as indicated by the Wagner–Nelson plots of percent drug absorbed over time. Multiple peaks in the pharmacokinetic profile were observed in subjects 12, 15, 24 and 30, and the time of the major peak correlated well with complete tablet disintegration times in the colon. A single extended peak was found in subjects 7 and 27 and absorption appeared to be complete before the tablet had fully disintegrated.

There was a large variation for the time of gastric emptying among individuals receiving formulation CD_2 but the time for colon arrival and the site for initial and complete disintegration was consistent. Mean pharmacokinetic data (serum concentrations over time) from subjects receiving CD_2 are presented in Figure 1. In six of the eight subjects who received CD_2, initial disintegration of the tablets was observed following colonic arrival (5.8 ± 2.3 h (range 2.9–10.5 h); see Table 2) post-dose. Multiple serum dexamethasone concentration peaks were observed in all eight subjects, and significant drug

Table 2. Disintegration profiles of the four formulations tested

Formulation	Initial tablet disintegration		Complete tablet disintegration	
	Post-dose (h)	Post-GE (h)	Post-dose (h)	Post-GE (h)
IR	0.17 ± 0.18*	—†	2.1 ± 5.2	2.0 ± 5.0
CD_1	1.7 ± 1.0‡	1.2 ± 1.3	7.9 ± 2.7§	6.1 ± 3.2
CD_2	5.8 ± 2.3¶	5.2 ± 2.3	12.4 ± 3.2**	7.4 ± 3.6
CD_3	3.6 ± 1.6††	2.7 ± 1.2	12.2 ± 3.8‡‡	8.3 ± 3.9

GE = gastric emptying.
* Data are means ± s.d. ($n = 8$).
† No discrete emptying of tablet observed due to disintegration in the stomach.
‡ Tablets disintegrated initially in the stomach ($n = 6$) or small intestine ($n = 2$).
§ Tablets disintegrated completely primarily in the ascending colon ($n = 6$), transverse colon ($n = 1$) or the splenic flexure ($n = 1$).
¶ Tablets disintegrated initially in the distal small intestine ($n = 2$), the ascending colon ($n = 4$), the transverse colon ($n = 1$) or descending colon ($n = 1$).
** Tablets disintegrated completely in the ascending colon ($n = 1$), hepatic flexure ($n = 2$), transverse colon ($n = 4$) or the descending colon ($n = 1$).
†† Tablets disintegrated initially in the distal small intestine ($n = 6$), the ileo-caecal junction ($n = 1$) or the ascending colon ($n = 1$).
‡‡ Tablets disintegrated completely in the ascending colon ($n = 3$), hepatic flexure ($n = 1$) or the splenic flexure ($n = 1$); the position of total disintegration was not observed in three of the subjects.

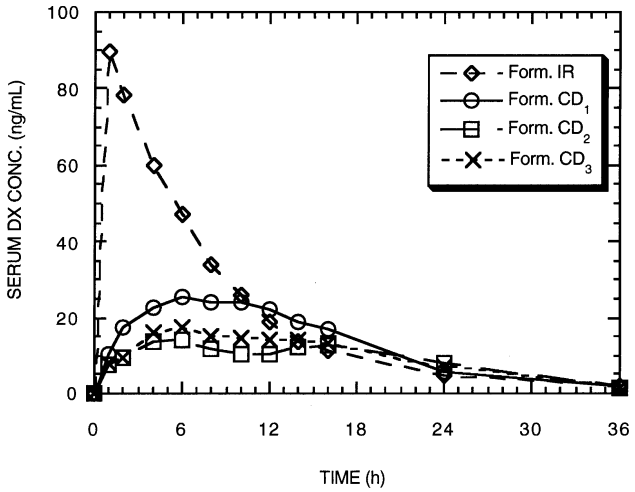

Figure 1. Mean serum concentrations of dexamethasone over time in subjects receiving formulations IR, CD_1, CD_2, or CD_3); $n = 8$ per formulation.

release was observed in the colon in subjects 3, 11, 18, 25 and 31.

The gastrointestinal transit and disintegration properties of formulation CD_2 are illustrated by a series of scintigraphic images from a representative subject in Figure 2.

Serum dexamethasone concentrations in subjects receiving formulation CD_3 are presented in Figure 1. Initial tablet disintegration (see Table 2) occurred 3.6 ± 1.6 h (range 2.3–7.4 h; in the small intestine in most cases) post-dose, with about 23% of the drug absorbed in the small intestine. In seven of the eight subjects receiving formulation CD_3, the tablets began to disintegrate in the small intestine, while in subject 5 disintegration commenced after the tablet reached the ascending colon. Complete tablet disintegration occurred 12.2 ± 3.8 h (range 5.9–14.9 h; $n = 5$) post-dose. In fact, complete disintegration did not occur in the first 16 h post-dose in three of the eight subjects who received formulation CD_3, and, in the remaining five subjects, disintegration occurred in the distal colon.

A summary of the pharmacokinetic parameters from all four formulations is shown in Table 3. The largest value of t_{max}, MRT, MAT and smallest value of C_{max} and AUC_{inf} can be found in formulation CD_3. All three CD formulations showed reduced *AUC* relative to the IR formulation. The relative bioavailability of the delayed-release preparations (CD_1, CD_2 and CD_3) to the IR formulation (excluding subject Nos 17 and 32) based on the mean AUC_{inf} were: 56.7% (CD_1), 37.9% (CD_2) and 44.0% (CD_3).

Statistical comparisons of the pharmacokinetic parameters ($AUC_{0,-t}$, AUC_{inf}, C_{max}, t_{max}, k_{el}, MRT_{oral} and $t_{1/2}$) were made between the IR formulation and the three CD formulations tested. When CD_1, CD_2 and CD_3 were compared individually to the IR formulation, a significant difference ($P < 0.05$) was observed for every comparison.

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