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Research Article

Colon-Specific Delivery of Dexamethasone from a Glucoside Prodrug in the Guinea Pig

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Dexamethasone-β-D-glucoside is a potential prodrug for colonic delivery of the antiinflammatory agent, dexamethasone. The ability of this prodrug to deliver dexamethasone selectively to the colon depends not only on its being slowly absorbed from the alimentary canal, but also on its having chemical and enzymatic stability in the stomach and small intestine. Once reaching the large bowel, it should be quantitatively hydrolyzed to release the active agent. The potential of dexamethasone-β-D-glucoside for colon-specific delivery of dexamethasone is assessed by determining the rates of its hydrolysis down the alimentary canal of the guinea pig, an animal in which an inflammatory bowel disease model has been developed. The hydrolytic activity is examined in tissues and luminal contents of the stomach, proximal and distal segments of the small intestine, cecum, and colon. For the tissues, the greatest hydrolytic activity is in the proximal small intestine, while the stomach, cecum, and colon have only moderate activity. In contrast, the contents of the cecum and colon show greater activity than the contents of the small intestine and stomach. The luminal contents retained β-glucosidase activity even after repeated centrifugation and resuspension in a buffer. The activity was unaffected by homogenization. These observations suggest that hydrolytic activity is associated with enzymes located on the surface of luminal cells. The movement and hydrolysis of dexamethasone-β-D-glucoside down the gastrointestinal tract of the guinea pig are also examined. About 20 to 30% of an oral dose appears to reach the cecum. Here the prodrug is rapidly hydrolyzed to the active drug. From intravenous administration of the prodrug and drug, it is apparent that dexamethasone-β-D-glucoside is poorly absorbed in the gastrointestinal tract (bioavailability, <1%). There is a ninefold selective advantage for delivery of dexamethasone in cecal tissues in the guinea pig under the conditions of this experiment. Thus, there is a potential for a decrease in the usual dose and a concomitant reduction in the systemic exposure to dexamethasone. Because humans have much less glucosidase activity in the small intestine, even greater site-selective delivery to the cecum and colon is expected.

KEY WORDS: colon-specific drug delivery; dexamethasone; dexamethasone-β-D-glucoside; intestinal microfloral-mediated drug hydrolysis; inflammatory bowel disease; prodrug; selective advantage.

INTRODUCTION

Colon-specific drug delivery has potential in the treatment of a variety of colonic diseases. The method is currently used to treat inflammatory bowel disease. Specifically, 5-aminosalicylic acid, an antiinflammatory agent, is delivered to the colon by oral administration of the prodrug, sulfasalazine (1,2). This system delivers active drug to the colonic tissue because the prodrug is poorly absorbed in the stomach and small intestine but releases drug in the colon. The release occurs by the action of azoreductase, an enzyme associated with the resident colonic bacteria (1,2). Other prodrugs containing 5-aminosalicylic acid are available in other countries or are being developed (3). Drug glycosides, which contain polar sugar moieties, have the potential to remain unabsorbed in the upper gastrointestinal tract but to release active drug via the action of bacterial glycosidases in the colon.

Steroidal antiinflammatory agents, when specifically delivered to the colon, may produce fewer and less intense systemic side effects than when administered orally or intravenously. This has been shown to be the case with rectal administration of corticosteroids in solution (1,4–6). The procedure can be helpful, but the drug generally only gains access to the rectum and descending colon. Unless the inflammation is restricted to this region, this method of specific delivery is not likely to be satisfactory.

The objective of this paper is to examine the potential of dexamethasone-β-D-glucoside to deliver the steroidal antiinflammatory agent, dexamethasone, to the colon. To this end, the extent of hydrolysis of dexamethasone-β-D-glucoside in the tissue and luminal contents of the gastrointestinal tract is examined. The time course of the distribution of prodrug and

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active drug down the alimentary canal after oral administration and a quantitative assessment of the selective advantage of this prodrug are also explored. As with sulfasalazine, no absorption in the stomach or small intestine, but complete release in the colon, is desired. Site-specific release is based on the greater occurrence of bacterial glycosidases in the colon compared with the small intestine (7).

Previous studies designed to test the concept of colon-specific drug delivery were performed in the rat (8,9), an animal with relatively high levels of bacterial glycosidase activity in its stomach and small intestine. In contrast, humans have low levels of glycosidase activity in these regions of the gastrointestinal tract (10,11). The guinea pig was chosen as the animal model for the present studies because the microbial distribution along its alimentary canal is more similar to that of the human than to that of the rat and it serves as an experimental model for inflammatory bowel disease (12).

METHODS

Materials

Dexamethasone-β-D-glucoside was prepared by a procedure described previously (8,9). All chemicals were HPLC grade or equivalent.

Animals

Male, Hartley guinea pigs (450–600 g, Charles River) were used. These animals, housed in the animal care facility, were used in three different kinds of experiments. In the first, the hydrolysis of dexamethasone-β-D-glucoside in homogenates of tissues and luminal contents of the gastrointestinal tract was examined. In the second, the release of dexamethasone down the gastrointestinal tract was studied *in vivo* in fasted animals. The animals were given free access to water but food was removed at 5:00 PM on the evening before the study. In the third set of experiments, animals were catheterized under anesthesia and dexamethasone-β-D-glucoside or dexamethasone was administered intravenously on the subsequent day and blood was sampled serially with time.

Hydrolysis *in Vitro*

Preparation of Homogenates

Studies were performed at 37°C to determine the rates of dexamethasone-β-D-glucoside (DG) hydrolysis in the tissues and contents of the various segments of the gastrointestinal tract. The guinea pigs were sacrificed by carbon dioxide anesthesia followed by bilateral thoracotomy. Each animal carcass was placed on a cold surface (aluminum foil on ice) and blood (about 20 ml) was immediately collected using intracardiac puncture. The gastrointestinal tract was removed from the carcass and chilled as quickly as possible. After removing the mesenteric and fatty tissues, the gastrointestinal tract was segmented into stomach, small intestine, cecum, and colon. The small intestine was further divided into proximal and distal segments of equal length. The luminal contents were removed by applying gentle pressure to the tissues. The individual tissues and luminal contents, placed on ice, were then weighed. The tissues were cut open longitudinally and washed with pH 7.4 isotonic phosphate buffer (1.90 g of KH_2PO_4, 8.10 g of Na_2HPO_4, and 4.11 g of NaCl to 1 liter of distilled water) to remove the remaining luminal contents. The tissues and luminal contents were homogenized with 2 vol of the isotonic phosphate buffer using a Brinkmann Polytron homogenizer.

Hydrolysis Conditions

The following hydrolysis conditions were used.

Stability in Solution. The stability of the prodrug was examined over 24 hr at 37°C in 0.1 *M* phosphate buffers, adjusted to pH 2.5, 6.0, and 7.5 to simulate the conditions in the human gastrointestinal tract (13). Samples of the incubated solution were removed at selected times during the 24-hr period and examined for extent of hydrolysis by high-performance liquid chromatography (HPLC) as described below.

Tissues and Luminal Contents. Duplicate samples of the homogenates, 250 μl in each test tube, were preequilibrated for 10 min at 37°C, using a Dubnoff metabolic shaking incubator. A 250-μl volume of buffer solution was used for all controls and zero-time samples. The metabolism was started by adding 50 μl of a 1 m*M* solution of dexamethasone-β-D-glucoside in 2% ethanol solution to the homogenates. Ethanol, required to dissolve the prodrug, had a final concentration of 0.33%. The reaction was stopped, at times predetermined by pilot experiments, by adding 1.0 ml of acetonitrile containing 2.8 μ*M* prednisone as internal standard. This procedure was shown, by adding substrate after acetonitrile, to stop the hydrolytic process in all the homogenates tested.

The final incubation concentration of dexamethasone-β-D-glucoside was 167 μ*M*. This concentration is considerably higher than that expected (30 μ*M* using a dilution volume of 1 liter) in the intestine of man following a usual upper daily dose of 9 mg in a single bolus dose. Concentrations less than 2 μ*M* could not be quantified because of variable interfering components in the contents of the intestine, particularly the large intestine.

Cecal content was further tested for the location and nature of the hydrolytic activity. Enzyme activity was measured under aerobic and anaerobic conditions as well as in the presence and absence of homogenization. Homogenization was performed without maintenance of anaerobic conditions. Anaerobic incubation conditions were simulated using an airtight cover over the metabolic shaking incubator into which nitrogen gas was flushed for 10 min while equilibrating the samples at 37°C and throughout the duration of the experiment. Aerobic conditions were achieved by exposing samples to room atmosphere. The cecal contents were homogenized with 2 vol of the isotonic phosphate buffer, while the nonhomogenized samples were prepared by gentle inversion of the tube containing the cecal contents and 2 vol of buffer. To determine if the activity is associated with luminal cells, nonhomogenized samples were further treated by centrifuging at 1000*g* and decanting the supernate. The pellet was resuspended with the same volume of buffer and the process was repeated three times.

Determination of Rate Constants. The rate constants for decomposition of the prodrug were obtained by linear regression of the natural logarithm of the concentration of dexamethasone-β-D-glucoside with time. The data showed pseudo first-order kinetics (straight line). The measured rate constant (slope) was multiplied by the factor (three) by which the original tissue or luminal content was diluted with isotonic phosphate buffer during homogenization under the assumption that enzyme activity is directly proportional to enzyme concentration. This rate constant, the value which presumably would have occurred had the sample not been diluted, is reported as well as the corresponding half-life.

Release of Dexamethasone in Gastrointestinal Tract

The guinea pigs were given a dose of 30 mg/kg of dexamethasone-β-D-glucoside (MW = 554.55, equivalent to 20 mg/kg of dexamethasone) prepared in 25% ethanol solution to a total volume of 1 ml. This prodrug solution was administered by gastric intubation, while the animal was carefully monitored for any signs of gastric intolerance to the solution. Animals, three or more per group, were sacrificed at 0.5, 1, 2, 3, and 5 hr by carbon dioxide anesthesia followed by bilateral thoracotomy.

Intravenous Administration of Prodrug and Drug

Animal Surgery

On the day before dosing, catheters made of silicone elastomer tubing (0.02-mm I.D.) were placed in the jugular vein and carotid artery of anesthetized (40 mg/kg sodium pentobarbital, 5% solution, ip) guinea pigs for dosing and removal of blood from the animal, respectively. The free ends of both catheters were externalized at the back of the neck. The lines were cleared and kept patent with an isotonic saline solution of heparin (50 units/ml).

Dosing and Sampling

A dose of 10 mg/kg of dexamethasone in 0.5 ml was prepared in 65% ethanol solution. This solution was delivered slowly into the animal over 5 min through the jugular vein. Twelve serial 0.5-ml blood samples were collected to determine the pharmacokinetic parameters. Other animals were sacrificed at 0.5, 1, 2, 3, 4, and 6 hr to estimate the time course of dexamethasone in the colon and cecum following intravenous administration. Upon sacrifice, dexamethasone concentrations in tissue and lumen were determined as in the recovery experiments after oral administration. A 500-μl sample of homogenate was used for analysis.

In a manner similar to that for dexamethasone, the disposition of dexamethasone-β-D-glucoside was also examined after intravenous administration. The prodrug (10 mg/kg) was administered in saline solution via a jugular vein catheter. Serial blood samples (0.5 ml) were obtained from the animals for measurement of prodrug in blood. These animals were kept in a metabolic cage for 24 hr to collect urine.

The doses given both orally and intravenously are considerably higher than those used therapeutically. The higher doses were used for two reasons. First, analytically it was necessary because of variable interfering peaks in cecal and colonic contents. Second, the concentration in the cecum after intravenous administration would not have been measurable at doses used clinically.

Selective Advantage

The selective advantage of oral administration of the prodrug was determined from measurement of dexamethasone in the cecum and blood after both intravenous administration of dexamethasone and oral administration of dexamethasone-β-D-glucoside. Observation of dexamethasone in both blood and the cecal and colonic tissues provides a relative measure of the exposure (area under concentration–time curve) in the tissue [AUC(tissue)] to that in blood [AUC(blood)] following the two methods of administration. The selective advantage (14) is then

$$\text{Selective advantage} = \left\{\frac{\text{AUC(tissue)}_{\text{p.o., prodrug}}}{\text{AUC(tissue)}_{\text{i.v., drug}}}\right\} \Big/ \left\{\frac{\text{AUC(blood)}_{\text{p.o., prodrug}}}{\text{AUC(blood)}_{\text{i.v., drug}}}\right\} \quad (1)$$

The numerator is the relative exposure, as measured by area, to the drug at the active site after oral administration of the prodrug and intravenous administration of the drug. The denominator is the relative drug exposure in the systemic circulation after the two administrations.

Data Analysis

The pharmacokinetic parameters of dexamethasone-β-D-glucoside and dexamethasone were determined after intravenous administration using a two-compartment model. The SIPHAR program (SIMED, Creteil, France) was used to fit the model parameters. Areas under the curves were estimated using the linear trapezoidal rule, and AUC ratios were used to calculate the bioavailability (15).

Analytical Methods

Dexamethasone-β-D-glucoside and dexamethasone were simultaneously determined by HPLC in body fluids, contents, and tissues. The chromatograph consisted of a Shimadzu LC-6A pump, Waters WISP 710B autosampler, Kratos SF 769Z detector, and Hewlett Packard 3390A integrator. The limit of detection upon injecting 100 μl of buffered solution was 1.0 μ*M*. The interday coefficients of variation were 11 and 5% for dexamethasone and 7.3 and 2.5% for dexamethasone-β-D-glucoside at 2 and 150 μ*M* concentrations, respectively.

An Altex Ultrasphere ODS, 5-μm, 4.6 mm × 150-mm column was used to separate dexamethasone, dexamethasone-β-D-glucoside, and prednisone (internal standard). A mobile phase flow rate of 1.8 ml/min was used. The mobile phase was 5% acetonitrile, 12% tetrahydrofuran, 5% methanol, and 78% 0.01 *M* KH_2PO_4 (1.36 g/liter). The retention times observed were prednisone (8.5 min), dexamethasone-β-D-glucoside (16.5 min), and dexamethasone (25 min).

Solutions (10 m*M*) of dexamethasone and dexamethasone-β-D-glucoside were prepared in 65% ethanol and 20%

ethanol, respectively. These solutions were combined and diluted to make 1 m*M* working solutions of the two substances. This solution was further diluted in isotonic phosphate buffer to make 100, 50, 25, 10, and 5 μ*M* solutions for preparation of standard curves. The internal standard (prednisone, 2.8 μ*M*) was prepared in acetonitrile and 1.0 ml of this solution was added to 250 μl of standards and buffered samples to simulate the conditions for arresting the hydrolytic activity in the homogenates.

Homogenates

All homogenates were treated identically. After adding 1 ml of acetonitrile containing prednisone, the samples were vortexed for 15 sec and centrifuged for 5 min. The entire upper clear layer was transferred to another test tube and saturated sodium chloride solution (0.5 ml) was added. Each tube was vortexed for 20 sec and centrifuged for 5 min. The acetonitrile-containing layer was transferred to another test tube, evaporated to dryness under nitrogen, and reconstituted with 180 μl of mobile phase. After vortexing, 100 μl was injected onto the HPLC column.

Although the dexamethasone peak occurred at 25 min, the samples containing the contents were run for 40 min to remove a substance with a retention time of 36 min. This peak and a peak with a retention time of about 8 min are apparently metabolites of the drug. They are not present unless dexamethasone-β-D-glucoside is administered to the animals and then they are not apparent until the 2-hr sample is taken. They did not occur in the incubations *in vitro*. The chromatograms of contents, especially of the small intestine and cecum, contained a number of peaks produced by polar endogenous compounds, and for this reason, concentrations below 2 μ*M* were not reliable and were not quantified.

Blood

The assay of dexamethasone had to be modified to measure the concentration in blood because a lower limit of detection was necessary. A Hamilton PRP-1 (10-μm, 4.6 mm × 150-mm) column was used to separate hydrocortisone (internal standard) from dexamethasone. A mobile phase flow rate of 1.8 ml/min was used. The mobile phase was 5% methanol, 12% tetrahydrofuran, 12% acetonitrile, and 71% 0.01 *M* KH_2PO_4 buffer (1.36 g/liter). The retention times were 7.0 min for hydrocortisone and 13.2 min for dexamethasone.

Dexamethasone and hydrocortisone were prepared in 1.0 m*M* solutions in ethanol. For dexamethasone, this solution was further diluted with isotonic buffer to make 100, 50, 25, 10, and 5 μ*M* standard solutions which, in turn, were diluted in blood to concentrations from 0.1 to 40 μ*M*.

All samples, standard curves in blood, and homogenates of tissue and luminal contents were treated identically. A 50-μl volume of 50 μ*M* solution of hydrocortisone (internal standard) was added to 500 μl of sample. After vortexing for 20 sec, 2.5 ml of pentane was added and the tubes were capped and rotated for 5 min. After centrifuging for 5 min, the top layer was discarded by pipetting. To the bottom layer was added 2.5 ml of a 75% ethyl acetate/25% pentane solution. The tube was again capped and rotated for 10 min and centrifuged for 10 min. The top layer was transferred to a test tube that contained 0.5 ml of a 0.5 *M* HCl solution. These samples were then rotated for 10 min and centrifuged for 10 min. The top layer was transferred to another tube and evaporated to dryness. After reconstituting with 100 μl of mobile phase and vortexing, the entire volume was injected into the chromatograph. The limit of sensitivity for this assay was 100 n*M* and the interday coefficients of variation at 0.25 and 20 μ*M* dexamethasone concentrations in blood were 9.8 and 5.6%, respectively. No interfering peaks were observed in the blood samples.

RESULTS

Hydrolysis *in Vitro*

The experiments were designed to assess the stability of dexamethasone-β-D-glucoside in solution and in the presence of the tissues and luminal contents of the guinea pig gastrointestinal tract. The prodrug was found to be stable in solution over 24 hr throughout the pH range, 2.5 to 7.5. Less than 1% of prodrug was lost after 24 hr at all of the pH values tested. Hydrolysis observed in the tissue and luminal content homogenates, therefore, must be due to factors other than pH. This observation and the ability of the protein precipitating agent, acetonitrile, to stop the reaction strongly suggest that hydrolytic enzymes (β-glucosidases) are responsible (7).

Typical semilogarithmic plots of the concentration of prodrug with time in the tissue and luminal content homogenates of the proximal small intestine and cecum are shown in Fig. 1. The rate constants for all the tissue and content homogenates tested are listed in Table I.

The observed distribution of hydrolytic activity in the luminal contents was that generally expected (7). The cecum, a large segment of the gastrointestinal tract in the guinea pig (16), and the colon had the most activity; the least activity was observed in the stomach (Table I). In contrast, the β-glucosidase activity in the tissue homogenates was greatest in the proximal small intestine, with the distal small intestine in second place. The activities in the gastric, cecal, and colonic tissues were an order of magnitude smaller.

The effect of homogenizing the cecal contents on the hydrolysis rate constant is shown in Fig. 2. Also shown are the effects of anaerobic and aerobic conditions during measurement of the hydrolysis rate constants. There was no difference statistically as determined by analysis of variance among any of the experimental groups.

Release of Dexamethasone in Gastrointestinal Tract

The movement of dexamethasone-β-D-glucoside down the gastrointestinal tract, as assessed by the amount in both tissues and luminal contents, is shown in Fig. 3. During the sojourn down the tract the prodrug is hydrolyzed to the active drug dexamethasone. The amount of dexamethasone present at these sites at various times is shown in Fig. 4. The recoveries of dexamethasone-β-D-glucoside and dexamethasone, expressed as a percentage of the dose administered, are listed in Table II. The total recovery in the gastrointestinal tract and the percentage of the dose in the cecum and colon are given in Table III.

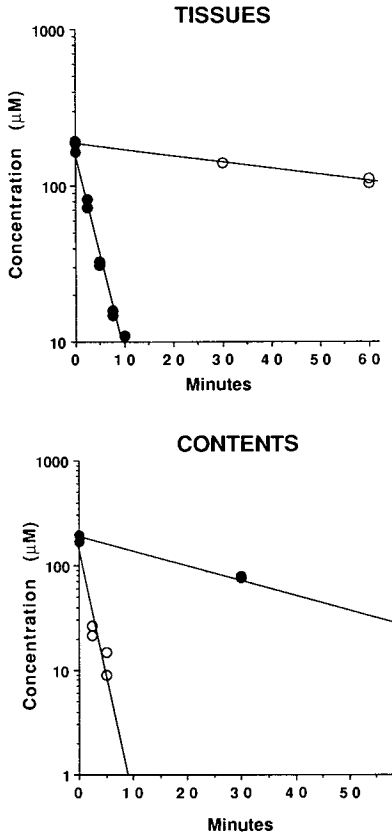

Fig. 1. Typical plots showing the concentration (duplicate values) of dexamethasone-β-D-glucoside remaining with time in homogenates of tissue (upper) and luminal contents (lower) in the proximal small intestine (●) and cecum (○) of a single animal. The lines are obtained by least-squares linear regression.

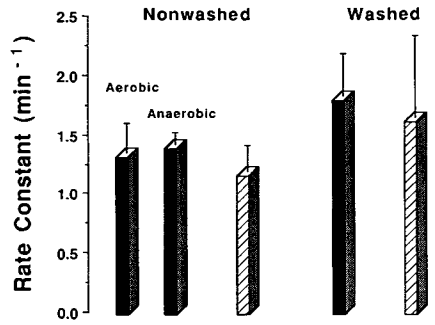

Fig. 2. Effect of various conditions on the rate constant for the hydrolysis of dexamethasone-β-D-glucoside in cecal contents expressed as values before dilution with 2 vol of isotonic phosphate buffer, pH 7.4. Two sets of experiments, represented by nonwashed and washed, were conducted. In the first set, the contents were homogenized (dark bars) or gently suspended (light bars) in an isotonic phosphate buffer solution. The homogenized samples were incubated under aerobic and anaerobic conditions. In the second set, after homogenizing the contents, the mixture was centrifuged and the precipitated pellet was resuspended in buffer solution. This process was repeated three times in samples initially prepared by homogenizing (dark bar) or gently resuspending (light bar) the contents. Mean values and standard deviations (error bars) of at least three experiments, each measured in duplicate, are shown.

Dexamethasone-β-D-glucoside quickly moves down the gastrointestinal tract in guinea pigs (Fig. 3). Within 30 min, about 20% of the dose was in the distal small intestine. The prodrug always reached the cecum and colon by 2 hr as seen by the appearance of dexamethasone at these sites (Fig. 4 and Table III). Compared to similar studies in the rat (8), transit in the small intestine was relatively rapid in the guinea pig. This difference in transit is consistent with other published data (17). The recovery of the dose in both the tissues and the luminal contents of the gastrointestinal tract falls off with time (Table II), but as expected, the recovery in the cecum and colon as a percentage of the total increases with time. Dexamethasone-β-D-glucoside accounts for most of the prodrug and drug recovered at 0.5 and 1 hr in both the luminal contents and the tissues (Table III). Conversely, dexamethasone accounts for most of that recovered from 2 hr onward.

Table I. Rate Constants of Dexamethasone-β-D-glucoside Hydrolysis at 37°C in Homogenates of the Luminal Contents and Tissues of the Gastrointestinal Tract of Guinea Pigs

	Contents	Tissues
Stomach	<0.01[a] (>100)	0.052 ± 0.017[b] (13)
Proximal small intestine	0.043 ± 0.063 (16)	0.60 ± 0.25 (1.2)
Distal small intestine	0.11 ± 0.05 (6.6)	0.44 ± 0.09 (1.6)
Cecum	1.34 ± 0.26 (0.5)	0.054 ± 0.025 (13)
Colon	1.70 ± 1.36 (0.4)	0.046 ± 0.010 (15)

[a] Not accurately measurable in the 90-min incubation interval.
[b] Rate constant (mean ± SD, n = 4 to 6) in units of min^{-1} in undiluted sample. Half-life (min) is in parentheses.

Intravenous Administration of Prodrug and Drug

The time course of dexamethasone-β-D-glucoside and dexamethasone in blood after intravenous administration of each compound is shown in Fig. 5. The decline of dexamethasone concentration was essentially monoexponential. Selected kinetic parameters of the two compounds are summarized in Table IV. The bioavailability (F) of dexamethasone from administration of the prodrug is 1.12; in contrast, the bioavailability of the prodrug is very small ($F < 0.01$). The selective advantage, calculated from Eq. (1), is 9.2 in cecal tissues. Pharmacokinetic parameters used to calculate the selective advantage are shown in Table V.

DISCUSSION

Hydrolysis *in Vitro*

Experiments were designed to assess the stability of the β-D-glucoside of dexamethasone in the gastrointestinal tract of the guinea pig *in vitro*. Ideally, the prodrug should be stable in the contents of the stomach and small intestine.

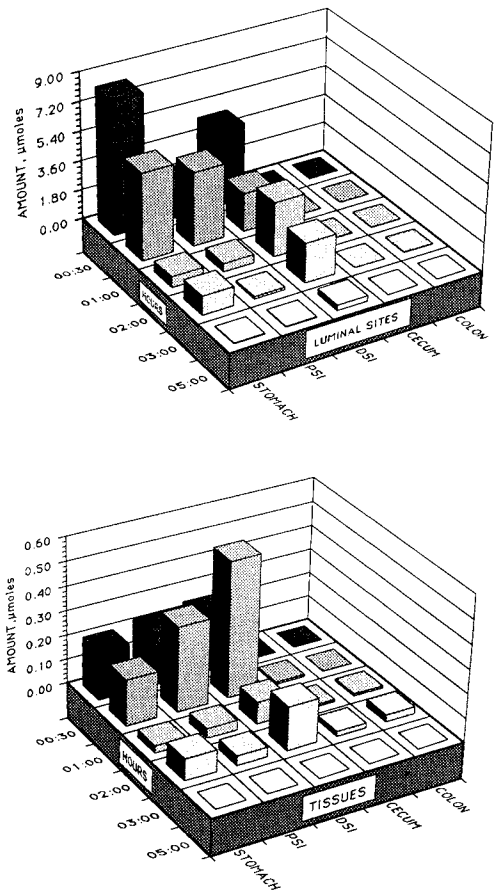

Fig. 3. The amount of dexamethasone-β-D-glucoside in the luminal contents (upper) and tissues (lower) of the gastrointestinal tract of the fasted guinea pig at various times after gastric intubation of 30 mg/kg. The prodrug moves quickly down the tract (dexamethasone always appears in cecum and colon within 2 hr; see Fig. 2), but the prodrug is virtually never measured in the cecum or colon. This observation is a result of rapid hydrolysis at these sites (1).

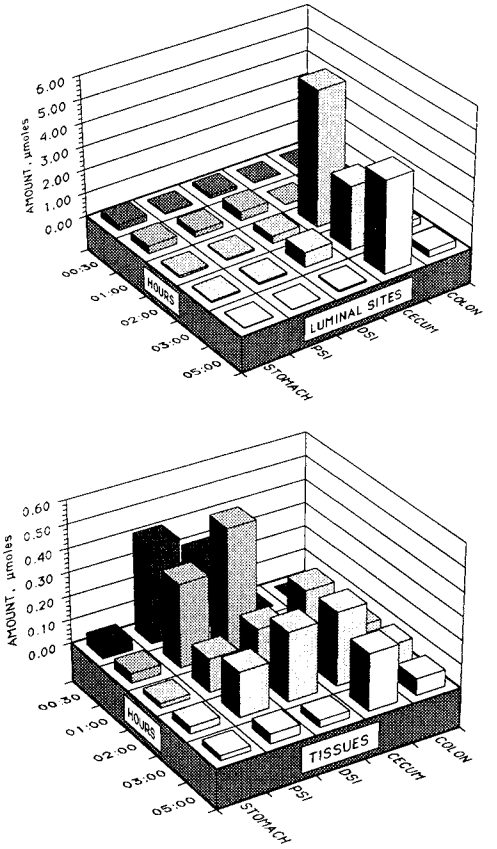

Fig. 4. The amount of dexamethasone formed and remaining in the luminal contents (upper) and tissues (lower) of the gastrointestinal tract of fasted guinea pigs at various times after gastric intubation of 30 mg/kg of the prodrug. Dexamethasone appears in the lumen of the large intestine by 2 hr. The drug appears in the tissues of both the large and the small intestines, a result of high hydrolytic activity in the contents of the cecum and colon.

However, when the prodrug reaches the large intestine, it should be quantitatively hydrolyzed to the active drug; the drug should then be absorbed by the colonic mucosa. The presence of mammalian, as well as bacterial, β-glucosidase activity in the gastrointestinal tract is well-known (18–22). Therefore, we examined the stability of the prodrug in the intestinal tissues as well as the intestinal contents. The greater enzyme activity observed in the tissue of the small intestine relative to that of the cecum, colon, and stomach is in agreement with results previously reported (18).

Fasting animals were used initially but the volume of contents recovered, especially from the small intestine, was insufficient to perform hydrolysis studies. In the fed animals, the hydrolysis tended to be far more variable in the contents than in the corresponding tissue homogenates. Differences in food and water intake and the subsequent dilutions and growth of the microflora undoubtedly contribute to much of the observed variability.

The calculated half-lives of dexamethasone-β-D-glucoside in the contents of the proximal and distal regions of the small intestine would appear to be too short to allow much of the prodrug to reach the colon in the guinea pig. Nevertheless, at least 20 to 30% of an oral dose of the prodrug given by oral intubation reaches the cecum. The number of bacteria in the small intestine is smaller by two or three orders of magnitude in humans than in the guinea pig, which results in a lower β-glucosidase activity, as shown in Table VI. Thus, the majority of a dose of dexamethasone-β-D-glucoside may reach the ileocecal valve without

Table II. Total Recovery of Dexamethasone-β-D-glucoside and Dexamethasone in the Entire Gastrointestinal Tract of the Guinea Pig After Gastric Intubation of Prodrug

Time (Hours)	Dose (μmol)	Total recovery (percentage of dose, mean ± SD)[a]	Recovery in cecum and colon (percentage of total recovery, mean ± SD)[a]
0.5	28.7	65 ± 4	0
1.0	29.6	52 ± 10	0
2.0	27.9	43 ± 4	51 ± 19
3.0	32.5	27 ± 8	33 ± 16
5.0	29.5	18 ± 3	97 ± 2

[a] n = 3 or 4.

Table III. Recovery of Dexamethasone-β-D-glucoside and Dexamethasone in the Gastrointestinal Tract of the Guinea Pig After Gastric Intubation of Prodrug

Time (hr)	Percentage of dose (mean ± SD)[a]	
	Dexamethasone-β-D-glucoside	Dexamethasone
Contents		
0.5	53 ± 4	1.6 ± 0.3
1.0	42 ± 5	3.1 ± 0.6
2.0	16 ± 12	25 ± 8
3.0	11 ± 4	16 ± 2
5.0	0.1 ± 0.2	16 ± 4
Tissues		
0.5	9.4 ± 9.1	2.5 ± 1.8
1.0	4.2 ± 3.0	3.6 ± 2.9
2.0	0.3 ± 0.2	2.7 ± 0.8
3.0	0.3 ± 0.2	3.2 ± 2.6
5.0	0.0 ± 0.0	1.2 ± 0

[a] n = 3 or 4.

undergoing hydrolysis. Additional information on the hydrolytic activity in the human gastrointestinal tract is needed before this prodrug can be applied in human therapeutics.

There is evidence that at least one other metabolite is formed in the alimentary canal. As shown in Fig. 6 for the contents of the cecum, the disappearance of dexamethasone-β-D-glucoside is not completely balanced by the formation of dexamethasone. Furthermore, incubation of dexamethasone in the same contents showed a slow decline in its concentration. This decline may account for the incomplete recovery of dexamethasone-β-D-glucoside and dexamethasone. In addition, there is evidence of the appearance of a small peak in the chromatogram with a retention time of 36 min as noted under Methods.

As most of the hydrolytic activity in the luminal contents is probably due to enzymes produced by the microflora of the gastrointestinal tract, homogenization and the attendant cell lysis may increase the activity normally present *in vivo* as a result of liberation of the hydrolytic enzymes. Cell lysis should give the prodrug free access to intracellular as well as extracellular enzymes. On the other hand, the hydrolytic enzymes may be degraded by lysosomal enzymes released by homogenization. The latter possibility was addressed by examining the hydrolysis in cecal and colonic contents immediately following homogenization and again after 2.5 hr of storage at room temperature. No demonstrable change in enzyme activity was observed. The former possibility was examined by measuring the hydrolytic activity after homogenization and after gently suspending the cecal contents in isotonic phosphate buffer. The absence of any difference between the two treatments (Fig. 2) suggests that the majority of the hydrolytic activity is located extracellularly or, if located intracellularly, penetration of prodrug into the bacterial cells is not the rate-limiting step. The location of the enzyme was tested by repeatedly (three times) centrifuging the cells and cellular debris, discarding the supernatant solution, and resuspending the pellet in isotonic phosphate buffer. Similar activities before and after the washing treatment suggest that the enzymatic activity is associated with the cells and that the prodrug either rapidly penetrates the bacterial cell or the activity resides on the surface of the cell.

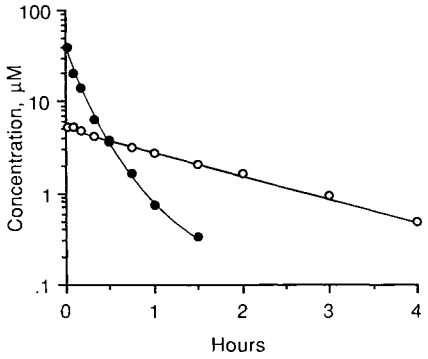

Fig. 5. Examples of the time courses of the blood concentrations of dexamethasone-β-D-glucoside (●) and dexamethasone (○) after intravenous administration of 10 mg/kg dexamethasone equivalents to different animals. Note the more prominent multiple exponential behavior of the prodrug.

The hydrolytic activity of bacterial cells is likely to be on the surface, as previous studies (18–21) have shown that β-glucosidase activity is located on the brush border of mucosal cells. Further, both bacterial and mucosal β-glycosidases probably are responsible for the observed overall activity. However, it is not clear how much intact and sloughed (22) mucosal cells contribute, relative to the bacterial cells, to the total activity (23).

The lack of a difference in activity of the cecal and colonic contents between aerobic and anaerobic conditions indicates that the enzymes do not depend on oxygen. Storage of the contents in the two different environments over extended periods of time could, of course, produce differences in the composition of the microflora and the corresponding activities.

Release of Dexamethasone in Gastrointestinal Tract

It has been known for many years that the bacteria residing in the large intestine of most mammals produce a variety of enzymes capable of hydrolyzing glycosides (24–26). Occasionally, toxic substances are released in the large intestine through the liberation of aglycones from certain plant glycosides, such as amygdalin and cycasin (25,27). Bacterial glycosidases have also been implicated in the activation of sennosides and related compounds in the large intestine (28).

In these studies, the prodrug, dexamethasone-β-D-glucoside, was administered by gastric intubation to guinea pigs. The time course of the distribution and hydrolysis of the prodrug in the gastrointestinal tract was then determined from measurement of the levels of dexamethasone-β-D-glucoside and dexamethasone in both tissues and luminal contents. To calculate the selective advantage for delivery to the guinea pig cecum, an intravenous dose of dexamethasone was administered. Ideally, the localized release of dexamethasone in the large intestine should lead to elevated cecal tissue levels. These levels should be greater than those from a systemic (in this case, intravenous) dose of dexamethasone. Furthermore, additional benefit should be obtained if the area under the dexamethasone blood concen-

Table IV. Pharmacokinetic Parameter Values (Mean ± SD) of Dexamethasone-β-D-glucoside and Dexamethasone

Intravenous administration						
Terminal half-life (hr)	Mean residence time (hr)	Volume of Distribution (L/kg)		Clearance (L/hr per kg)	Fraction excreted unchanged	Oral administration, bioavailability
		Terminal phase	Steady state			
			Dexamethasone-β-D-glucoside			
0.26 ±0.02	0.27 ±0.02	0.76 ±0.40	0.57 ±0.25	2.00 ±0.91	0.97 ±0.11	<0.01[a]
			Dexamethasone			
1.82 ±0.18	2.58 ±0.28	1.43 ±0.33	1.43 ±0.32	0.55 ±0.13	0.20[b]	1.12

[a] Negligible quantities of dexamethasone-β-D-glucoside were detected in blood.
[b] Only one animal in this group was kept in the metabolic cage for 24 hr.

tration–time curve after oral intubation of the prodrug is less than that after an equivalent intravenous dose of the drug.

The prodrug/drug composition at each site down the gastrointestinal tract after oral administration varies, as shown in Figs. 3 and 4. The primary factors influencing the ratio of dexamethasone-β-D-glucoside and dexamethasone are the glucosidase activity and the absorption of dexamethasone-β-D-glucoside and dexamethasone into the body. Our previous studies *in vitro* show that the β-glucosidase activity in the lumen of the cecum and colon is much higher than in the stomach or intestine. Indeed, the activity in the luminal contents of the cecum and colon is so great that it is not surprising that dexamethasone-β-D-glucoside is virtually never measured at these sites.

The presence of β-glucosidase activity in the contents of the small intestine suggests that the entire dose of a glucoside prodrug cannot be delivered quantitatively to the colon. While the overall delivery of dexamethasone does not appear to be high in the guinea pig, the probability of being more successful in humans is high. The β-glucosidase activity in the small intestine is well over 100-fold less in man (7), while the activity in the colon is comparable (7,24), as shown in Table VI.

Dexamethasone-β-D-glucoside and dexamethasone have different mean residence times in the body (Table IV). This difference is primarily a result of the much higher renal clearance of the prodrug. Indeed, virtually all prodrug elimination occurs renally as shown by the 97% recovery of the prodrug in urine. These results indicate that urinary recovery of prodrug is a good measure of the amount reaching the systemic circulation following an oral dose. Dexamethasone, reported to be primarily metabolized (29), has a longer mean residence time because it is more slowly eliminated; in this study only 20% of an intravenous dose was recovered unchanged in the urine.

The bioavailability of dexamethasone from oral administration of the prodrug is approximately 100%, while that of the prodrug is negligible. Thus, the decreasing recovery of the dose with time in the gastrointestinal tract after oral administration of the prodrug (Table II) must be associated primarily with hydrolysis to dexamethasone and its subsequent absorption.

Some of the dexamethasone-β-D-glucoside may be hydrolyzed in the tissues of the gastrointestinal tract. One would expect hydrolysis at this site to require absorption of the prodrug. Such transport may not be necessary, as β-glucosidase activity has been shown to occur in the brush border of the mammalian small intestine (18–20,30). Our studies *in vitro* demonstrate comparable β-glucosidase activity in both homogenized and nonhomogenized guinea pig

Table V. Data Used to Calculate Selective Advantage for Delivery of Dexamethasone to the Guinea Pig Cecum[a]

Dose (mg/kg)	Route	Site	$AUC^{Dexamethasone}_{0\rightarrow 6\ hr}$ ($\mu M \cdot$ hr)
Dexamethasone-β-D-glucoside (30)[b]	p.o.	Cecum	210.37
Dexamethasone-β-D-glucoside (30)	p.o.	Blood	23.64
Dexamethasone (10)	i.v.	Cecum	9.51
Dexamethasone (10)	i.v.	Blood	9.91

[a] $n = 3$.
[b] 30 mg/kg dexamethasone-β-D-glucoside = 20 mg/kg dexamethasone.

Table VI. Estimated Bacterial β-Glucosidase Activity in the Proximal Small Intestine (PSI), Distal Small Intestine (DSI), and Large Intestine of Humans and Four Laboratory Species[a]

Animal species	β-Glucosidase activity[b]		
	PSI	DSI	Cecum/colon
Human	0.0001	0.004	78
Rabbit	0.02	0.5	44
Guinea pig	0.04	1.0	78
Rat	3.6	16	94
Mouse	17	49	120

[a] Estimated activity based on data from Ref. 7.
[b] Micromoles *p*-nitrophenol-β-D-glucoside degraded per hour per gram intestinal contents.

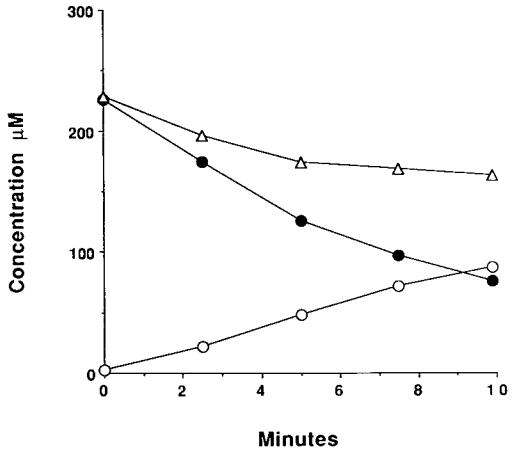

Fig. 6. Disappearance of dexamethasone-β-D-glucoside (●) and appearance of dexamethasone (○) during incubation with a typical homogenate of the contents of distal small intestine. The sum of the molar concentrations (△) declines with time, suggesting that either dexamethasone-β-D-glucoside or dexamethasone is converted to another product.

cecal contents, indicating that dexamethasone-β-D-glucoside may not need to cross bacterial cell membranes to reach the site of hydrolysis in the luminal contents. The contribution of enzymes in the tissue and luminal contents *in vivo* is unknown, but the almost-total lack of availability of prodrug ($F < 0.01$, Table III) suggests that the prodrug is not absorbed.

The selective advantage of oral administration of the prodrug was calculated to be about 9.2 in the cecal tissues. Had the fraction of the dose reaching the colon (about 20–30% observed) been greater, as may occur in humans, the selective advantage would have been even greater. Thus, there is a potential for a decrease in a usual dose and a concomitant reduction in the systemic exposure to this antiinflammatory agent. Indeed, we have observed that the oral dose of dexamethasone-β-D-glucoside prodrug needed for equivalent efficacy is lower than that for dexamethasone (31). Attempts to gain a selective advantage by the use of enemas has been only partially successful. An antiinflammatory agent so administered may gain access only to the rectum and descending colon (1). The prodrug approach here is probably more likely to be advantageous for treating inflammatory lesions in the ascending and transverse colon. A combination of the two approaches may be the best tactic in some inflammatory bowel disease conditions.

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