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Synthesis and chemical stability of glucocorticoid-dextran esters: potential prodrugs for colon-specific delivery [1]

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Summary

Dextran ester prodrugs have been proposed as a means of delivering drug to the colon. In this study, methylprednisolone and dexamethasone were covalently attached to dextran (M_w = 72 600) by the use of a succinate linker. In addition, dexamethasone was attached by glutaric acid to investigate the effect of linker molecule on hydrolysis kinetics. The kinetics of degradation of the hemiesters and corresponding dextran conjugates were measured as a function of pH and temperature. Intramolecular migration of the linker molecule from the 21- to the 17-position on the glucocorticoid occurred in all three hemiesters, although to a greater extent in methylprednisolone-hemisuccinate. The dextran conjugates were also incubated at 37°C, pH 6.8 and the chemical degradation half-lives were as follows: dexamethasone-succinate-dextran 75 h; dexamethasone-glutarate-dextran 103 h and methylprednisolone-succinate-dextran 82 h. Incubation of dexamethasone-21-hemisuccinate with rat gastrointestinal (GI) tract luminal contents indicated that the hemiester is hydrolyzed throughout the GI tract. Greatest esterase activity, however, was found in the small intestine. By contrast, little drug (< 3%) was released from dexamethasone-succinate-dextran during incubation with small intestinal contents despite the high esterase activity. Dexamethasone and dexamethasone-21-hemisuccinate were released at faster rates during incubation with cecum and colon contents. This combination of chemical stability and selective enzyme-mediated drug release in the large intestine indicates that these dextran prodrugs have potential in colon-specific delivery of glucocorticoids.

Introduction

Chronic colitis, e.g., ulcerative colitis and Crohn's disease, are presently treated with anti-inflammatory drugs (Hanauer and Kirsner, 1988). The side effects of steroid therapy may be reduced by selectively delivering drug to the colon (Friend et al., 1991; Tozer et al., 1991). One potential way of doing so is to attach the drug to dextran, a polar macromolecule that prevents drug absorption in the small intestine. Previous reports on colon-specific delivery using dextran prodrugs have focused on non-steroidal antiinflammatory agents, which are directly coupled to dextran using the drugs' carboxylic acid groups

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(Harboe et al., 1988; Larsen and Johansen, 1989b). Experiments with these conjugates have demonstrated that dextranases and esterases in the colon degrade the conjugate, and liberate drug at this site (Harboe et al., 1989a,b; Larsen et al., 1989a, 1991a,b).

Antiinflammatory glucocorticoids do not possess carboxylic acid groups and first must be chemically transformed in order to react with dextran. Various spacer molecules have been previously used in the synthesis of drug-polysaccharide conjugates, namely glucocorticoid-carbonates (Khue and Galin, 1985; Khue et al., 1986) benzyl alcohol-carbonate (Weibel et al., 1991) and metronidazole-hemiesters (Larsen et al., 1988). In this study, dexamethasone and methylprednisolone were attached to dextran using succinic acid. Dexamethasone-glutarate conjugate also was made to determine whether a longer spacer has an effect on drug release. Succinic and glutaric acids were chosen because they have been previously used in the synthesis of metronidazole-dextran conjugates. Hemiester spacers have not, however, been tested in colon-specific dextran prodrug mediated drug delivery. Structures of the conjugates are shown in Fig. 1.

The chemical stability of these conjugates was studied as a function of pH at 60°C. Methylprednisolone-21-hemisuccinate has been previously demonstrated to undergo an acyl migration reaction, forming the 17-hemisuccinate (Anderson and Taphouse, 1981; Anderson et al., 1984). This phenomenon was studied for the dexamethasone hemiesters. The steroid-hemiesters and dextran conjugates were incubated at 37°C in isotonic buffer at pH 6.8 to measure the chemical degradation expected to occur in vivo. In addition, dexamethasone-succinate-dextran was incubated with rat gastrointestional (GI) tract contents to demonstrate the magnitude of enzyme-mediated drug release at different sites down the GI tract.

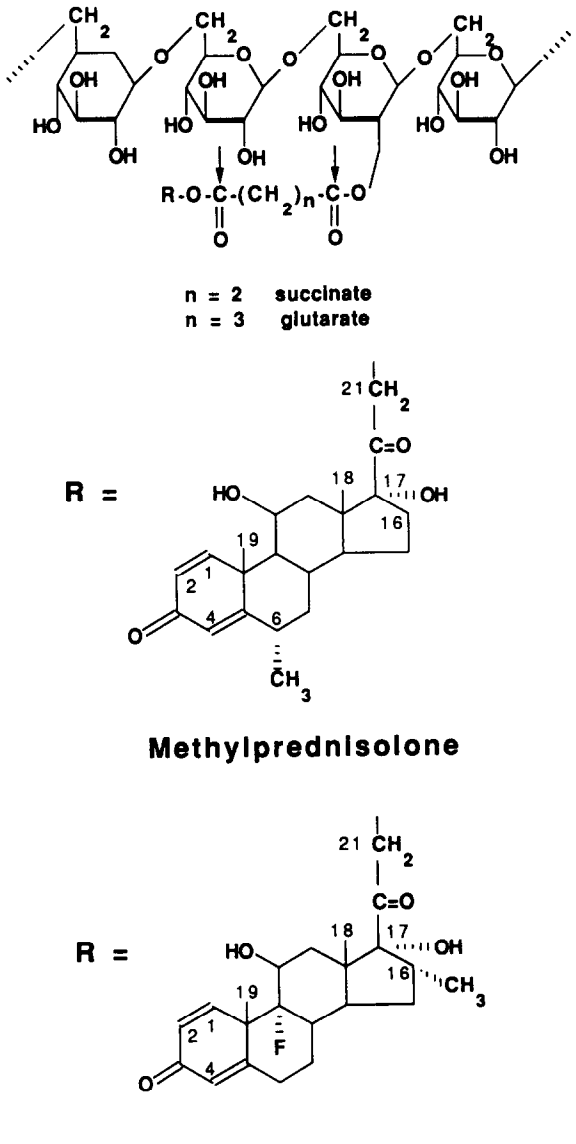

Fig. 1. Chemical structures of the dextran conjugates. Arrows indicate possible sites of ester hydrolysis.

Materials and Methods

Methylprednisolone (MP), methylprednisolone-21-hemisuccinate (MPS) and dexamethasone (D) were generous gifts from Upjohn (Kalamazoo, U.S.A.). Dextran (weight-average molecular weight = 72 600; number-average molecular weight = 43 400) was obtained from Sigma (St. Louis, U.S.A.). 4-Dimethylaminopyridine (DMAP), 1,1'-carbonyldiimidazole, succinic anhydride and glutaric anhydride were all obtained from Aldrich (Milwaukee, WI). Acetonitrile, acetone, diethyl ether, methanol, methylene chloride

and dimethylsulfoxide (DMSO) were of HPLC grade.

Apparatus

High-performance liquid chromatography (HPLC) was performed on the following equipment: Shimadzu SCL-6A system controller, Shimadzu LC-6A pumps, Waters WISP 710B autoinjector, Shimadzu SPD-6AV variable wavelength detector and a Hewlett Packard HP3396A integrator. An Orion model 231 pH meter was used for all pH measurements. ^{1}H-NMR spectra of the compounds in d_6-DMSO solutions at room temperature were obtained on a General Electric QE-300 (300 MHz) NMR spectrometer, and referenced to 0.1% w/v TMS at 0 ppm.

Synthesis of dexamethasone-21-hemiesters

Dexamethasone-21-hemisuccinate (DS) was prepared by a previously published method (Vermeersch et al., 1985) with the following modifications. Dexamethasone, succinic anhydride and 4-dimethylaminopyridine (12.7 mmol of each) were dissolved in 400 ml anhydrous acetone. After reacting for 30 min at 25°C the acetone was removed by vacuum evaporation in a rotating flask. Dexamethasone-21-hemisuccinate was obtained in the monohydrate form by recrystallization from ethanol/water (29:71). The number of moles of water of crystallization was determined by weight loss after drying at 115°C for 15 h. m.p., 209–213°C. ^{1}H-NMR: δ (ppm): 0.787 (d, 3H, 16-CH_3), 0.879 (s, 3H, 18-CH_3), 1.487 (s, 3H, 19-CH_3), 2.499 (s, 4H, succinate C2 and C3), 6.008 (s, 1H, C4-H), 6.226 (d, 1H, C2-H), 7.287 (d, 1H, C1-H), 12.252 (broad, 1H, succinate COOH). Analysis: Calc. for $C_{26}H_{33}FO_8$: C, 61.17; H, 6.91; N, 0. Found: C, 61.33; H, 6.92; N, 0.

Dexamethasone-21-hemiglutarate (DG) was also synthesized by the same method (Vermeersch et al., 1985), however, 19 mmol glutaric anhydride was used. After removal of the acetone, the residue was dissolved in 40 ml methanol. After the addition of 15 ml water, the white precipitate obtained was removed by filtration and discarded. The filtrate was diluted to 100 ml with water, and left at 4°C overnight to produce white crystals. Dexamethasone-21-hemiglutarate did not contain water of crystallization. m.p., 224–229°C. ^{1}H-NMR: δ (ppm): 0.790 (d, 3H, 16-CH_3), 0.886 (s, 3H, 18-CH_3), 1.489 (s, 3H, 19-CH_3), 1.771 (m, 2H, glutarate C3), 2.307 (t, 4H, glutarate C2 and C4), 6.008 (s, 1H, C4-H), 6.227 (d, 1H, C2-H), 7.295 (d, 1H, C1-H), 12.140 (broad, 1H, glutarate COOH).

Analysis: Calc. for $C_{27}H_{35}FO_8$: C, 64.02; H, 6.96; N, 0. Found: C, 63.93; H, 7.00; N, 0.

Thin-layer chromatography

The steroids and hemiesters were analyzed by thin-layer chromatography. Silica gel plates with fluorescent marker were used (250 μm thickness, K5F, Whatman, NJ, U.S.A.) and the mobile phase consisted of methylene chloride-diethyl ether-methanol-water (77:15:8:1.2). Spots were visualized by UV (254 nm). R_f values were as follows: dexamethasone, 0.43; methylprednisolone, 0.38; dexamethasone-hemisuccinate, 0.11; dexamethasone-hemiglutarate, 0.21; methylprednisolone-hemisuccinate, 0.09. These values are comparable to published data (Pharmaceutical Codex, 1979).

Synthesis of dextran conjugates

Dextran conjugates were synthesized using a modification of a previously published procedure (Harboe et al., 1988). All steps were performed in a glovebag under nitrogen. 5 mmol of the hemiester (MPS, DS or DG) was dissolved in 15 ml of anhydrous DMSO. Next, 11 mmol of 1,1'-carbonyldiimidazole was added, and reacted with the hemiester for 30 min. Dextran (200 ml of a 5% w/v solution in DMSO), and 17.5 ml of triethylamine were then added. The reaction was continued for 21 h at 25°C. The dextran conjugate was precipitated by adding 300 ml of ethanol-diethyl ether (50:50) to the DMSO solution while stirring. The liquid was discarded, and the gummy polymer was redissolved in 200 ml DMSO. The precipitation step was repeated, then the polymer was dispersed in ethanol using a Brinkmann homogenizer, fitted with a Kinematica rotor attachment. The polymer powder was washed twice with diethyl ether, and dried under a stream of dry nitrogen.

Drug content of the dextran conjugates was determined by measurement of glucocorticoid re-

leased after hydrolysis in 0.1 M NaOH. MPS-dextran, DS-dextran and DG-dextran contained 9.5, 7.4 and 4.3 mg glucocorticoid per 100 mg dextran conjugate, respectively.

Water solubility of the MPS-dextran, as expected for all the conjugates, decreased with an increasing amount of attached steroid. Poor water solubility was observed in MPS-dextran conjugates containing more than 14 mg glucocorticoid per 100 mg polymeric prodrug.

^{1}H-NMR of the dextran conjugates showed peaks of the glucocorticoid methyl groups and unsaturated carbon protons. There was complete absence of free carboxylate protons downfield at 12 ppm, indicating that the hemiesters were covalently attached to dextran. In addition, analysis by HPLC showed that the dextran conjugates contained less than 0.1% non-covalently bound drug by weight.

HPLC analysis

The glucocorticoids and hemiesters were measured by reversed-phase HPLC. The column (15 cm × 4.6 mm i.d.) contained a polystyrene-vinyl benzene copolymer (5 μm, PRP-1, Hamilton, Reno, U.S.A.). The mobile phase composition for the separation of MP, D, MPS and DS was 30% acetonitrile and 70% trisodium citrate buffer (20 mM) adjusted to pH 5.5 with phosphoric acid. For the analysis of DG, the buffer portion of the mobile phase was pH 5.65. In all cases, the flow rate was 1 ml/min with detection at 242 nm. In this HPLC method, dextran conjugates were eluted in the solvent front. The retention times of glucocorticoids and hemiesters were all less than 9.5 min.

Chemical stability at elevated temperature

Solutions of the hemiesters, or their respective dextran conjugates, were made in pH 7 buffer. This stock solution was added to preheated (60 ± 0.2°C) buffers to give a final concentration of 50 μM. All buffers were 0.1 M and adjusted to an ionic strength of 0.5 M by the addition of NaCl. This ionic strength was used in many previous studies of dextran conjugate hydrolysis kinetics (Larsen, 1986, 1989; Larsen and Johansen, 1987; Larsen et al., 1988b, 1989b). The buffer systems were as follows: hydrochloric acid (pH 1.4), citrate (pH 3), acetate (pH 5), phosphate (pH 7) and borate (pH 9). Samples were withdrawn at various times, and immediately chilled and adjusted to pH 3 to stop the reaction. The samples (200 μl) were then analyzed by HPLC. Rate constants were calculated using the initial rate method (Anderson and Taphouse, 1981). In these experiments a known concentration of reactant is incubated for a short period and the rate of product formation is measured. Generally less than 5% of the reactant is consumed, thus reactant concentration remains essentially constant throughout the incubation. The rate of product formation divided by the amount of reactant yields the rate constant. The rate constants k_{HYDRO} and $k_{21\rightarrow17}$ were measured by incubating 21-hemiester and measuring the production of steroid and 17-hemiester, respectively. The dextran hydrolysis rate constants k_{ESTER} and k_{STERD} were measured by incubation of the dextran conjugates and measuring the production of hemiester and steroid respectively. For dextran conjugates the concentration of glucocorticoid was calculated from the drug content of the conjugate multiplied by the conjugate concentration (e.g., for DS-dextran, which contains 7.4 mg dexamethasone per 100 mg conjugate, a 0.265 mg/ml solution is equivalent to 50 μM dexamethasone).

Incubations with rat GI tract contents and buffer

Male Sprague-Dawley rats (250 g) were used throughout these experiments. The rats were killed by decapitation and the GI tract was removed and chilled on ice. The contents of each tissue (stomach, proximal half of the small intestine (PSI), distal half of the small intestine (DSI), cecum and colon) were removed and diluted to 15% w/v with chilled isotonic buffer. Stomach contents were diluted with acetate buffer (pH 4.4) whereas phosphate buffer (pH 6.8) was used for all other contents. These are the pH values normally found in the rat GI tract (Smith, 1965). At the start of the incubation, 0.5 ml of reactant solution (buffer containing 1.5 mM glucocorticoid) was added to 1 ml of the 15% w/v GI tract content homogenate. Thus, the initial glucocorticoid concentration was equivalent to 500 μM and

the incubation solution contained 10% w/v GI tract content. The initial glucocorticoid concentration was 500 μM in order to obtain measurable rates of product formation in all tissues during a 160 min incubation period. Samples (200 μl) were withdrawn at predetermined intervals using a wide-bore pipet tip. Samples were added to chilled tubes containing 200 μl of saturated aqueous sodium chloride and 60 μl of 6% phosphoric acid. After addition of internal standard, the samples were extracted with methyl *t*-butyl ether/pentane (60:40) and the organic phase was removed and evaporated. The residue was dissolved in 100 μl of methanol, and 50 μl was analyzed by HPLC. The mobile phase consisted of 35% acetonitrile and 65% of 50 mM trisodium citrate adjusted to pH 4.1 with phosphoric acid. HPLC instrumentation was the same as previously described except that a 250 mm × 4.6 mm i.d. C_{18} column was used (Econosphere 5 μM, Alltech, Deerfield, IL). These chromatographic conditions were necessary in order to achieve separation from interfering peaks. It was not possible to resolve 17- and 21-hemiesters with this mobile phase due to its low pH, therefore acyl migration was not studied in these experiments.

As a direct measure of the chemical stability of the hemiesters and dextran conjugates at physiologic conditions, control incubations at pH 6.8, 37°C were also performed.

Results and Discussion

Synthesis

Vermeersch et al. (1985) described the use of 4-dimethylaminopyridine as an acyl transfer catalyst in the synthesis of metronidazole-hemisuccinate. These authors reported high yields (95%) using 0.05 mol of 4-dimethylaminopyridine per mol of metronidazole. When this method was used for the synthesis of dexamethasone hemiesters we obtained low yields (< 10%) even after 20 h at room temperature. Increasing the molar ratio of 4-dimethylaminopyridine to 1:1 gave yields of 94% for dexamethasone-21-hemisuccinate and 56% for dexamethasone-21-hemiglutarate.

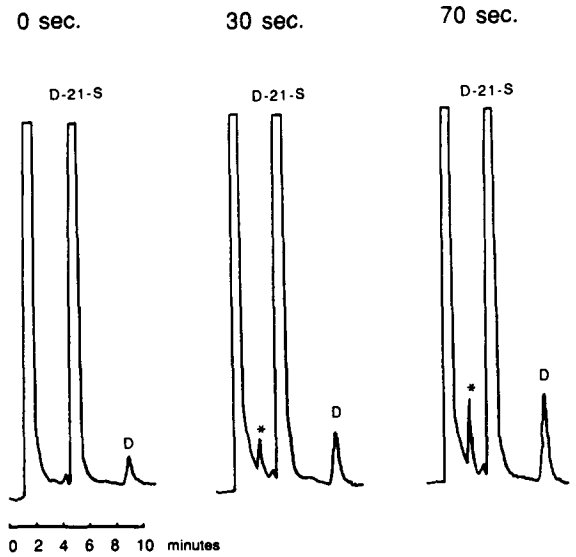

Fig. 2. HPLC chromatograms showing the hydrolysis products of dexamethasone-21-hemisuccinate (D-21-S initial concentration 50 μM, pH 9, 60°C), after 0, 30 and 70 s of incubation. The unknown peak (*) is postulated to be dexamethasone-17-hemisuccinate; the peak labeled (D) is dexamethasone.

The dextran conjugates were readily purified in high yields (> 90%). The purification method enabled the rapid production of large batches (10 g) of the conjugates.

Hydrolysis and acyl migration at 60°C

HPLC analysis of 21-hemiester incubation products showed, in each case, the formation of an early eluting peak, at approx. 3 min. Fig. 2 demonstrates that this peak increases with time. Based on previously published data, this peak was hypothesized to be a 17-hemiester (Anderson and Taphouse, 1981). The mobile phase containing this peak was collected and subjected to further alkaline hydrolysis. The unknown peak was found to produce 21-hemiester and subsequently to yield glucocorticoid. This sequence of events is in agreement with other reports (Anderson and Taphouse, 1981), and supports the model proposed in Fig. 3. Due to the unavailability of authentic 17-hemiesters, a single standard curve using 21-hemiester peak area was used to measure both 17- and 21-hemiesters. Molar absorptivity was assumed to be identical for the two hemiesters.

From the structures shown in Fig. 1, both the

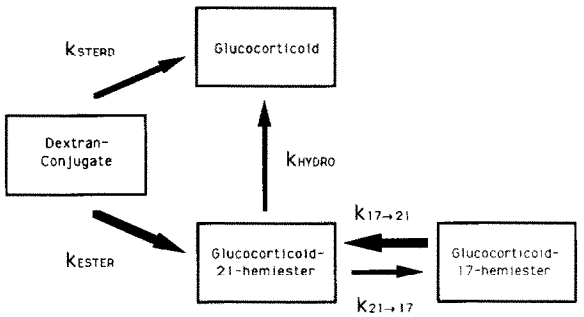

Fig. 3. Schematic model showing ester hydrolysis and acyl migration pathways. The thicknesses of the arrows indicate the relative magnitude of the rate constants.

dextran-linker and glucocorticoid-linker ester bonds are expected to be susceptible to hydrolysis. Therefore, during incubation of the dextran conjugates both drug and hemiester are released.

The model shown in Fig. 3 includes both ester hydrolysis and acyl migration reactions. Rate constants k_{ESTER} and k_{STERD}, measured after the incubation of dextran conjugates, describe the production of hemiester and glucocorticoid, respectively. The rate constant k_{HYDRO}, measured after the incubation of glucocorticoid hemiesters describes the production of glucocorticoid. The rate constants $k_{21\rightarrow17}$ and $k_{17\rightarrow21}$ quantify acyl migration to and from the 17-hemiester. The ratio of $k_{17\rightarrow21}$ and $k_{21\rightarrow17}$ is identical to the hemiester concentration ratio occurring at the time the 17-hemiester concentration peaks. At this time, the rates of formation and degradation are equal. Anderson and Taphouse (1981) have demonstrated using methylprednisolone-hemisuccinates that this ratio remains essentially constant in the pH range from 7 to 9. The ratios of $k_{17\rightarrow21}$ to $k_{21\rightarrow17}$ at pH 9 are as follows; MPS = 4.14 ± 0.01, DS = 69.78 ± 0.21 and DG = 91.01 ± 2.50 (all ratios are expressed as mean ± S.D., $n = 3$). These ratios indicate that acyl migration rate constants for conversion of glucocorticoid-17-hemiesters to the respective 21-hemiesters ($k_{17\rightarrow21}$) are considerably larger than the opposing acyl migration rate constants ($k_{21\rightarrow17}$), which accounts for the small amount of 17-hemiester observed during incubation.

Table 1 lists the rate constants at 60°C as a function of pH. In the ester hydrolysis reactions, i.e., k_{ESTER}, k_{STERD} and k_{HYDRO} the hydrolysis is faster at extreme pH values, and slowest at pH 3. In all cases, the rate of release of hemiester is faster than that of glucocorticoid. This difference is most pronounced for the dexamethasone conjugates. It has been postulated that adjacent hydroxyl groups on the dextran molecule catalyze the hydrolysis of the dextran-linker bond, thus making k_{ESTER} greater than k_{STERD} (Larsen, 1986). Ester hydrolysis rates are quite similar for DS and DG, in accord with work on hydrocortisone-hemiesters, (Garrett, 1962a,b) and metronidazole-hemiesters (Larsen et al., 1988a).

Acyl migration ($k_{21\rightarrow17}$) increases with pH, in agreement with published work on methylprednisolone-21-hemisuccinate (Anderson and Taphouse, 1981). Anderson et al. (1984) have postulated that a tetrahedral intermediate species is formed during $21 \rightarrow 17$ succinate migration in methylprednisolone-hemisuccinate. Dexamethasone possesses a 16α-methyl group, not present in methlyprednisolone, which may theoretically lessen the formation of the tetrahedral intermediate in dexamethasone, compared to methylprednisolone, hemiesters.

Fig. 4 shows the reaction products formed during the incubation of DG-dextran at pH 9, 60°C. Note the rise and fall of the two hemiglutarates accompanied by the accumulation of dexamethasone. Dexamethasone-17-hemiglutarate was present in low concentrations throughout the incubation.

Hydrolysis in buffer under physiologic conditions

The chemical hydrolysis of glucocorticoid-hemiesters and dextran conjugates proceeds slowly at pH 6.8, 37°C (Table 2). In agreement with the elevated temperature data k_{ESTER} is always larger than k_{STERD}. Furthermore, k_{HYDRO} is larger for methylprednisolone-21-hemisuccinate (0.204 day^{-1}) than for the two dexamethasone-hemiesters (DS = 0.063 day^{-1} and DG = 0.025 day^{-1}).

To determine the overall chemical stability of the dextran conjugates, a degradation rate constant (k_{DEGRAD}) was defined;

$$k_{DEGRAD} = k_{ESTER} + k_{STERD}$$

TABLE 1

Rate constants for ester hydrolysis and acyl migration at 60°C as a function of pH

pH	MPS		DS		DG		MPS-dextran		DS-dextran		DG-dextran	
	$k_{21\rightarrow17}$ (day^{-1})	k_{HYDRO} (day^{-1})	$k_{21\rightarrow17}$ (day^{-1})	k_{HYDRO} (day^{-1})	$k_{21\rightarrow17}$ (day^{-1})	k_{HYDRO} (day^{-1})	k_{ESTER} (day^{-1})	k_{STERD} (day^{-1})	k_{ESTER} (day^{-1})	k_{STERD} (day^{-1})	k_{ESTER} (day^{-1})	k_{STERD} (day^{-1})
1.4	n.d [a]	0.72 (19) [b,c]	n.d.	0.65 (6)	n.d.	1.09 (5)	0.32 (2)	0.38 (2) [d]	0.46 (4)	0.43 (7)	1.09 (10)	1.25 (9)
3	0.04 (6)	0.07 (37)	0.02 (12)	0.07 (6)	0.01 (19)	0.03 (25)	0.01 (37)	0.01 (45)	0.13 (16)	0.01 (20)	0.03 (11)	0.02 (20)
5	0.49 (4)	0.12 (15)	0.14 (15)	0.12 (4)	0.02 (9)	0.03 (16) [d]	0.06 (22)	0.05 (17)	0.07 (6)	0.03 (3)	0.03 (10) [d]	0.01 (21)
7	1.71 (16)	0.81 (5) [d]	0.34 (8)	0.45 (15)	0.20 (3)	0.30 (8)	1.60 (7)	1.00 (8)	2.20 (2)	0.94 (3)	1.80 (6)	0.77 (11)
9	38.02 (5)	33.26 (7)	7.20 (11)	19.50 (10)	8.29 (23)	17.11 (27)	67.39 (11)	50.98 (4)	94.61 (7)	46.80 (6)	74.45 (10)	38.86 (14)

[a] Not detectable.

[b] Mean of three replicates.

[c] Numbers in parentheses are coefficients of variation (percent).

[d] Mean of two replicates.

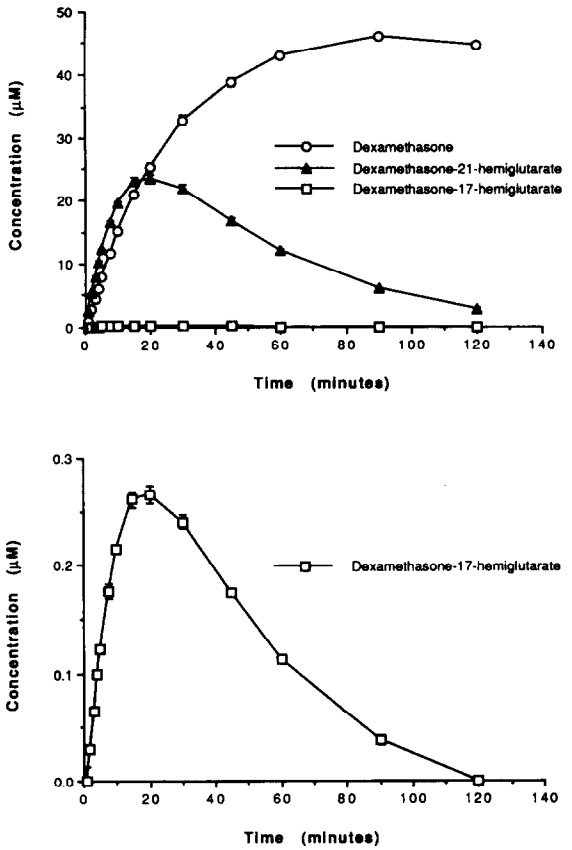

Fig. 4. Kinetics of the reaction products after incubation of DG-dextran at pH 9, 60°C. Dexamethasone-17-hemiglutarate concentrations are shown in the lower panel. Data are means ± S.E. ($n = 3$).

This parameter was used to calculate the chemical stability half-life ($t_{1/2}$) at pH 6.8, 37°C. The values are as follows: MPS-dextran, 82 h; DS-dextran, 75 h and DG-dextran, 103 h.

TABLE 2

Rate constants for ester hydrolysis in buffer under physiologic conditions (pH 6.8, 37°C)

	k_{ESTER} (day^{-1})	k_{STERD} (day^{-1})	k_{HYDRO} (day^{-1})
Dextran-MPS	0.113 ± 0.009 [a]	0.091 ± 0.007	0.204 ± 0.012
Dextran-DS	0.152 ± 0.012	0.069 ± 0.006	0.063 ± 0.007
Dextran-DG	0.124 ± 0.015	0.037 ± 0.002	0.025 ± 0.002

[a] Mean ± S.D., $n = 5$.

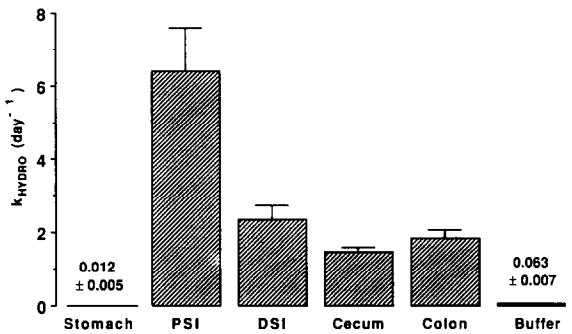

Fig. 5. Dexamethasone-21-hemisuccinate hydrolysis rate constant (k_{HYDRO}) measured in GI tract contents. PSI, proximal small intestine; DSI, distal small intestine. Data are means ± S.E. ($n = 5$).

Hydrolysis of DS and DS-dextran by rat GI tract contents

The bar graph in Fig. 5 shows that dexamethasone-21-hemisuccinate is more rapidly hydrolyzed in the proximal small intestine (PSI) than further down the GI tract. The stomach and buffer show negligible activity. Carboxyesterase distribution along the GI tract has been shown to have a similar distribution (Hänninen et al., 1987)

The release of drug-hemiester and drug from DS-dextran is exemplified by Fig. 6. In these 160 min incubations with 10-fold dilutions of GI tract contents, less than 3% of the polymer-bound drug is released as drug and hemiester in upper GI tract contents, whereas 14% is released in large intestine contents. These results imply that the dextran backbone protects drug-polymer ester bonds from hydrolysis by small intestinal esterases.

The length of time the dextran conjugate spends in each segment of the GI tract also determines the extent of drug release. In humans, the small intestinal transit time is generally 2–3 h (Davis et al., 1986) whereas colonic transit time ranges from 13 to 68 h (Hardy et al., 1985). After oral ingestion the conjugates may be retained for a sufficient period of time in the large intestine for drug release to be quantitative.

The combination of chemical stability, selective enzyme-mediated drug release and the long residence time in the human colon indicates that

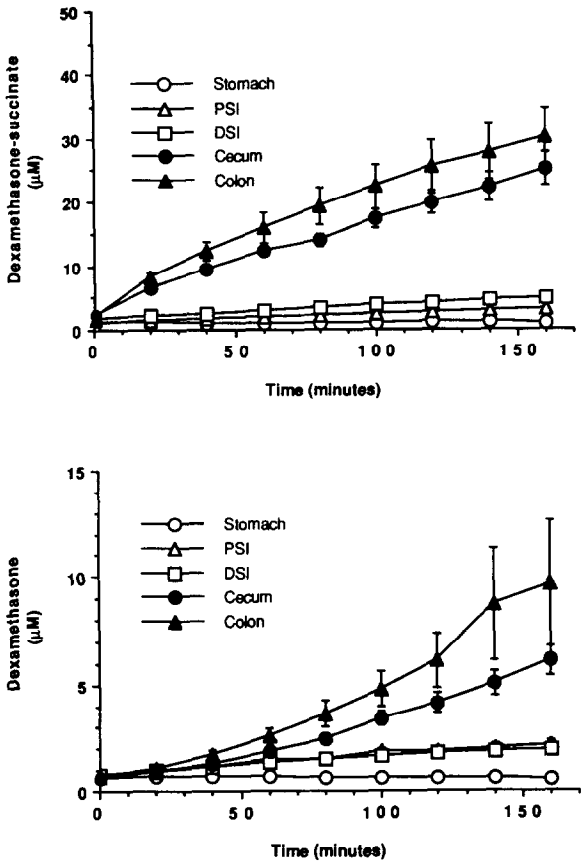

Fig. 6. Release of dexamethasone-succinate (upper panel) and dexamethasone (lower panel) during incubation of dexamethasone-succinate-dextran in GI tract contents. Data are means ± S.E. ($n = 5$).

these conjugates have potential in colon-specific delivery of glucocorticoids.

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