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Research Article

Macromolecular Prodrugs. XV. Colon-Targeted Delivery—Bioavailability of Naproxen from Orally Administered Dextran–Naproxen Ester Prodrugs Varying in Molecular Size in the Pig

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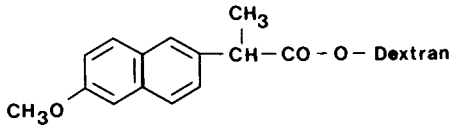
The bioavailability of naproxen after oral administration of aqueous solutions of various dextran–naproxen ester prodrugs in pigs was determined. The dextran prodrugs employed ranged in molecular weight from 10,000 to 500,000. As calculated relative to an equivalent oral dose of parent naproxen, the absorption fractions of all the derivatives were close to 100%. Only small interindividual variation of naproxen bioavailability was observed. The naproxen plasma profiles for all the administered prodrugs exhibited a characteristic lag time of naproxen appearance in the blood (2–3 hr). Compared to administration of the prodrugs alone, coadministration of excess of the parent dextran further delayed the absorption of naproxen from the GI tract. The results of the present study demonstrate the potential of dextran prodrugs for colon site-specific delivery of drugs containing a carboxylic acid functional group.

KEY WORDS: dextran ester prodrugs; naproxen bioavailability; oral colon-targeted delivery.

INTRODUCTION

Various aspects related to the design of oral drug delivery systems have been reviewed (1–3). Most of the recent research efforts have been devoted to the development of delivery systems providing systemic sustained drug action, whereas positioned drug release formulations have received far less attention. For several drugs the proximal part of the small intestine constitutes a specific absorption window. Improved bioavailability of such compounds incorporated in hydrodynamically balanced (4) and bioadhesive delivery systems (5) has been observed due to delayed gastric emptying of the latter formulations. The bioadhesive approach has also been exploited to accomplish local drug action in the stomach (6). Various pathological conditions of the large bowel warrant site-specific drug delivery to the colon as discussed later in this article.

In previous studies we have administered orally a dextran T-70-naproxen ester prodrug (I) to rabbits (7) and pigs (8). In the former case an absorption fraction of naproxen of 0.66 was found, whereas the bioavailability of the drug in the pig was close to 100%. The results of preliminary experiments indicated that drug regeneration took place in the GI tract below the ileum. The present study was undertaken in order to determine the bioavailability of naproxen after oral administration of dextran-naproxen prodrugs varying in molecular weight in the pig. In an associated paper (9) studies pertinent to the elucidation of the mechanism of the drug activation in homogenates of various segments of the pig GI tract are reported.



Scheme I

EXPERIMENTAL

Materials

Naproxen [(+)-2-(6-methoxy-2-naphthyl)propionic acid] was obtained from Sigma, St. Louis, Mo. The dextran fractions T-10 (M_w 10,300; M_n 4900), T-40 (M_w 40,700; M_n 15,300), T-70 (M_w 74,000; M_n 36,000), and T-500 (M_w 488,000; M_n 184,800) were purchased from Pharmacia, Sweden. M_w and M_n refer to the weight and the number average molecular weights of the dextrans, respectively. The dextran–naproxen ester prodrugs were synthesized as previously reported (10) and characterized according to our earlier studies (11,12). The degree of substitution has been ex-

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pressed as the percentage of milligrams of naproxen released per milligram of the conjugate. Methanol used in the mobile phase was of chromatographic grade. All other chemicals were of analytical or reagant grade.

Apparatus

The HPLC equipment consisted of a Hitachi L-6200 Intelligent pump, a Hitachi L-4000 variable wavelength detector, a Rheodyne Model 7125 injection valve, a Hitachi 655A-40 auto sampler, and a Hitachi D2000 chromatointegrator. The column used, 125 × 4.6 mm, was packed with Spherisorb ODS-1 (5-μm particles), PhaseSep, U.K. During chromatography the column was protected by a small precolumn packed with Perisorb RP-8 (30- to 40-μm particles), Merck, F.R.G. The pH readings were done with a Radiometer Type pHM 26 meter at the temperature of study.

Bioavailability Studies

Seven female pigs (Danish land race/Yorkshire) from an SPF production herd, weighing 30 to 45 kg, were used. They were housed in individual pens, kept at a 12:12-hr light/dark cycle at 22°C. The pigs were fed twice a day with a standard pelleted diet and provided with water ad libitum. Insertion of polyurethane catheters for blood sampling was carried out as previously described (8). The animals were fasted overnight prior to peroral drug administration, while water was allowed ad libitum. An aqueous solution of the dextran conjugates (corresponding to 1 mg free naproxen/ml) was given mixed with 300 g of food. The pigs were fed in connection with each blood sampling. A solution of parent naproxen adjusted to pH 7.5 with sodium hydroxide was administered likewise. Due to the limited solubility of naproxen the concentration of the latter solution was only 0.5 mg ml^{-1}. All doses given corresponded to 3.6 mg naproxen equivalents/kg body weight.

Analytical Procedure

Blood sampling was done as previously reported (8). A 100-μl plasma sample was deproteinized with 300 μl methanol. The mixture was vortexed and centrifuged at 10,000*g* for 3 min. The supernatant was prepared for HPLC analysis by using an autosampler. A mobile phase composed of methanol–0.02 *M* phosphate buffer, pH 2.5 (65:35, v/v), was employed. The flow rate was maintained at 1 ml min^{-1} and the column effluent was monitored at 271 nm. Naproxen plasma samples were treated likewise and were used as an external standard.

RESULTS

Using the pig as an animal model the bioavailability of naproxen was determined after oral administration of a solution of the individual dextran-naproxen ester prodrug. Conjugates ranging from 10,000 to 500,000 Da of the carrier dextran were employed. In all cases doses corresponding to 3.6 mg parent naproxen/kg body weight were given to each of the three pigs. During the 4 days between the experiments the animals were at rest and fed a standard pelleted diet.

The area under the curve was calculated from the equation

$$AUC_{0-\infty} = AUC_{0-t} + C_t/k_e \quad (1)$$

where C_t represents the plasma concentration at time t and k_e is the apparent first-order elimination rate constant derived from log-linear regression of the terminal part of the curve. AUC_{0-t} was obtained by using the trapezoidal rule.

The individual absorption data obtained after oral administration of the dextran prodrugs are presented in Table I. Taken into account that the derivatives were given mixed with food, a good correlation of the AUCs between the animals is observed. The low AUC of pig C (given the T-40 conjugate) might have been associated with the development of an acute infection which was treated with antibiotics. Also, the shapes of the obtained plasma concentration versus time profiles of the three pigs were quite similar. A representative example is depicted in Fig. 1. The T-10, T-70, and T-500 conjugates were administered to the same group of pigs, whereas the T-40 prodrug was given to three new pigs. In Table II are summarized the relative bioavailability of naproxen from the various conjugates. The absorption fractions were calculated as the ratio AUC(prodrug)/AUC(naproxen p.o.) using each animal as its own control. No apparent trend is observed between the *F* values and the variation in weight of the pigs. It is seen that the interindividual differences of the average bioavailability of naproxen from the three dextran prodrugs are surprisingly small, and in all cases the relative absorption fractions are close to 100%.

As discussed previously, using rabbits (7) and pigs (8) as animal models pure hydrolytic release of naproxen from the dextran prodrugs cannot account for the initial phase of systemic drug absorption. In the pH range 1–7.4 the pH-dependent hydrolytic half-lives exceed 180 hr (10,14). Consequently, one or more enzyme systems residing in the GI tract are involved in the drug liberation. After incubation in different biological media (plasma, synovial fluid from an inflamed joint and liver homogenates) insignificant esterase-facilitated hydrolysis of dextran esters of metronidazole di-

Table I. Pharmacokinetic Parameters of Naproxen After Oral Administration of Ester Prodrugs Derived from Dextran T-10 (I), Dextran T-40[a] (II), and Dextran T-500 (III) in Pigs (All Doses Given Were Equivalent to 3.6 mg Free Naproxen/kg Body Weight)

	Pig		
Parameter	A	B	C
I (DS 7.1)[b]			
T_{max} (hr)	16.5	10.0	16.5
C_{max} (μg ml^{-1})	15.4	14.2	16.7
AUC (μg ml^{-1} hr)	475.9	406.7	544.2
II (DS 6.9)			
T_{max} (hr)	16.1	11.6	11.6
C_{max} (μg ml^{-1})	17.4	16.8	12.7
AUC (μg ml^{-1} hr)	637.8	603.1	284.1
III (DS 6.8)			
T_{max} (hr)	15.7	23.8	15.7
C_{max} (μg ml^{-1})	10.6	10.9	13.2
AUC (μg ml^{-1} hr)	392.6	454.0	420.4

[a] A new group of pigs was employed.
[b] DS, degree of substitution.

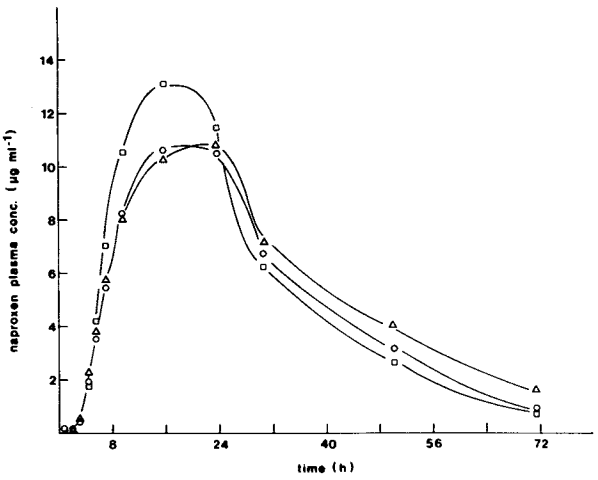

Fig. 1. Naproxen plasma concentration–time profiles after oral administration of a solution of a dextran T-500 prodrug (DS 6.8) corresponding to 3.6 mg naproxen per kg body weight in pigs. (○) 35 kg; (△) 33 kg; (□) 35.5 kg.

carboxylic acid hemiesters (13) and NSAID compounds (14) was observed, suggesting that in the case of drug fixation close to the matrix, steric protection of enzymatic attack is a general property of the dextran carriers. Therefore, it was obvious to suggest that drug activation from the conjugates in the GI tract initially involves partial depolymerization of the dextran backbone effected by dextran splitting enzymes (dextranases) (9). Sufficiently small fragments might in turn be substrates for various hydrolases located in the GI tract. To test this hypothesis each of the three pigs was given orally a dextran T-10 conjugate (3.6 mg naproxen equivalents/kg body weight). Two of the pigs were additionally administered 10 and 20 g of parent dextran T-10, respectively. From Fig. 2 it is seen that coadministration of parent dextran results in an enhancement of the lag time of naproxen appearance in the systemic circulation, with the 20-g dextran dose affording the most delayed absorption profile. On the other hand, the AUC values calculated for the three pigs were of comparable size. These observations might reflect that the presence of excess of parent dextran in the GI tract retards the fragmentation of the dextran prodrug, probably due to a limited capacity of the dextranase system. In an associated paper (9) experiments have been carried out providing substantial support for the above mentioned hypothesis. The results have further documented that drug regeneration occurs selectively in the cecum and colon of the pig.

The average naproxen plasma concentration versus time curves obtained after administration of the T-10, T-40, T-70, and T-500 conjugates are depicted in Fig. 3, and in Table III are presented the various average pharmacokinetic parameters. In nearly all cases the individual experimental results deviated less than 30% from the presented average values. The bioavailability of naproxen from all the prodrug derivatives was high as calculated relative to both i.v. and p.o. administration of the parent drug. The ratio AUC (naproxen p.o.)/AUC(naproxen i.v.) amounted to approximately 0.9, which is quite similar to the value found in man (15). The plasma elimination rate constants, k_e, calculated after administration of the dextran T-70 and T-500 prodrug appear to be slightly lower compared to the values found after administration of the parent drug. Probably the naproxen absorption phase from the latter prodrugs is extended, which might explain the observed difference. On the average, the half-life of the terminal phase (14.4 hr) agrees well with those in man after i.v. injection [13.9 hr (15)] and p.o. administration of naproxen [17 hr (16)], respectively. Although no statistical analysis has been carried out, the data suggest that the average naproxen plasma concentration–time profiles of the prodrugs differ in the time of peak level, T_{max}, and the maximum drug plasma concentration, C_{max}. The lower molecular weight conjugates give rise to a more rapid naproxen appearance in the blood and a higher plasma level, as expected from the hypothesis that the smaller prodrugs are depolymerized faster by dextranases than the higher molecular weight derivatives (9).

DISCUSSION

The average absorption characteristics of naproxen presented in Table III demonstrate clearly that the pharmacokinetic parameters related to oral administration of the parent drug and the dextran prodrugs differ significantly. Whereas naproxen per se is absorbed rapidly, T_{max} for the dextran T-500 conjugate corresponds to 18 hr. In addition, the prodrug plasma profiles exhibit a characteristic lag time (2–3 hr) of naproxen appearance in the blood. Since the major sites of drug regeneration from the dextran derivatives in the pig GI tract are the cecum and the colon, the observed enhancement of T_{max} for the conjugates might be attributed to two different mechanisms. (A) After ingestion the prodrug molecules may reach the colon at different times. Little is known about the gastric emptying rate in the pig, but re-

Table II. Bioavailability of Naproxen from Various Dextran Ester Prodrugs, Administered Orally, Calculated Relative to Naproxen p.o., Using Each Animal as Its Own Control

Pig	Naproxen p.o. AUC (μg ml^{-1} hr)[a]	F%			
		Conjugate T-10	Conjugate T-70	Conjugate T-500	Average
A	458.6 (43.5 kg)	103.8 (40 kg)	83.3 (44.5 kg)	85.6 (35 kg)	90.9
B	450.5 (42 kg)	90.3 (36.5 kg)	78.8 (41 kg)	100.8 (33 kg)	90.0
C	407.3 (40 kg)	133.6 (40 kg)	112.2 (44 kg)	103.2 (35.5 kg)	118
		X = 109.2	X = 91.4	X = 96.5	

[a] From a previous study (8).

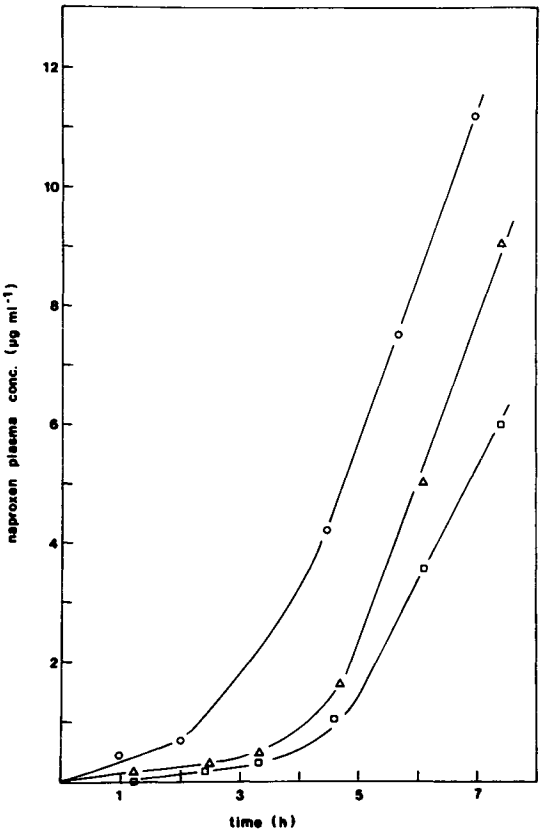

Fig. 2. Initial naproxen plasma profiles after oral administration of a dextran T-10 prodrug (DS 7.1) corresponding to 3.6 mg naproxen per kg body weight. (○) Prodrug alone; (△) prodrug + 10 g dextran T-10; (□) prodrug + 20 g dextran T-10.

cently we have performed experiments showing that after 2 hr a considerable amount of the administered prodrug dose was still in the stomach, whereas at 4 hr the conjugates were detectable primarily in the cecum and the colon (8). The

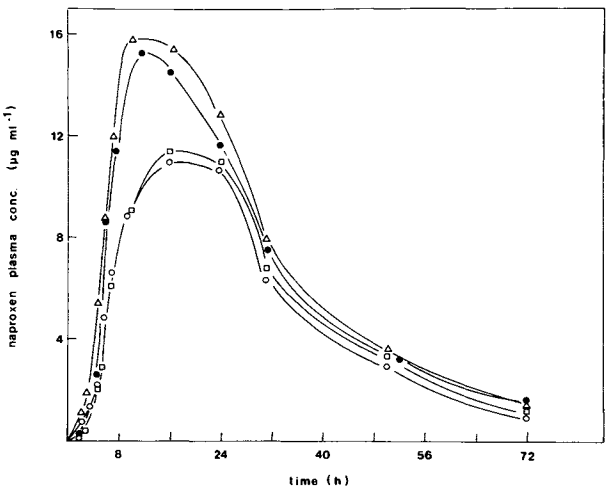

Fig. 3. Average naproxen plasma concentration–time curves from three pigs after oral administration of solutions of dextran prodrugs varying in molecular size (all doses corresponded to 3.6 mg naproxen per kg body weight). (□) M_w 500,000 (DS 6.8); (○) M_w 70,000 (DS 8.2); (●) M_w 40,000 (DS 6.9); (△) M_w 10,000 (DS 7.1).

dextran conjugates were given as aqueous solutions and fast clearance from the stomach was to be expected. The observed relatively long residence time in the stomach may therefore most likely be due to the coadministration of solid food. Although the latter preliminary experiments do not allow an unequivocal conclusion to be drawn, they indicate that the small intestine transit time of the prodrugs is of the order of magnitude of 2 hr. This value correlates well with those reported for various chemical entities in man [3 ± 1 hr (1)]. (B) With the above discussion in mind it appears probable that cleavage of the dextran chains constitutes the rate-limiting step in the absorption of naproxen after the prodrug administration. Results of the present study indicate that the large bowel dextranase system has a limited capacity. Similarly, in colon homogenate experiments (9) the initial rate of naproxen formation decreased with increasing parent dextran added to the reaction mixtures. Saturating the dextranase system could serve to distribute the prodrug molecules throughout the ascending and transverse colon before releasing the therapeutic agent; previously it was difficult to convey drug compounds to the latter parts of the colon with conventional formulations.

There are various colonic disorders warranting delivery of effective amounts of drug compounds selectively to the diseased site. Recently it has been suggested that the large bowel may offer an opportunity for systemic absorption of insulin and other peptide drugs (17,18). Technological approaches to site-specific colon drug delivery include capsules coated with an acrylic-based resin (19) and peptide drugs imbedded in polymers cross-linked with azoaromatic groups (17). The ability of the gut microflora to hydrolyze glycosides has formed the basis for the design of steroid glycoside prodrugs intended for colon-positioned delivery (20,21). In rats up to 60% of an oral dose of the prodrugs was found to reach the cecum (20). The prodrug sulfasalazine has long been used in the management of colon inflammatory disorders like ulcerative colitis. After passage through the small intestine the active species, 5-aminosalicylic acid, is released from the prodrug after cleavage of the azo-bond by action of azo-reductases secreted from colonic bacteria (22). However, disadvandages of sulfasalazine therapy include systemic adverse reactions, which have been attributed to the sulfapyridine promoiety (23). A more elegant prodrug of 5-ASA, azodisal sodium, has been developed, consisting of two molecules of 5-ASA linked together by an azo-bond (24). The prodrug is poorly absorbed in the small intestine and generates quantitatively the active species in the colon. Another promising 5-ASA prodrug, requiring bioactivation through azo-reductases in the colon, is a water-soluble polymer (25). Based on experiments in rats, the potential advantages of this high molecular weight prodrug, which contains 5-ASA residues linked at the 5 position by an azo-bond to an inert polymer backbone containing sulfanilamide spacer arms, were suggested to encompass nonabsorption/nonmetabolism in the small intestine, positioned 5-ASA activation in the large bowel and no adverse reactions to the parent nondigestible polymeric carrier.

Using the pig as an animal model (8,9; present study), we have demonstrated the potential of dextran prodrugs for site-specific delivery of drugs containing a carboxylic acid functional group. The prodrugs possess several properties

Table III. Bioavailability of Naproxen After Oral Administration of Dextran–Naproxen Ester Prodrugs in Pigs[a] (Average Pharmacokinetic Parameters, Determined After Administering Solutions of Dextran Prodrugs Varying in Weight Average Molecular Weight, in Comparison to Those Obtained after p.o. and i.v. Administration of Solutions of Parent Naproxen)

Compound[b]	C_{max} ($\mu g\ ml^{-1}$)	T_{max} (hr)	k_e (hr^{-1})	AUC ($\mu g\ ml^{-1}\ hr$)	AUC (p.o.) × 100/ AUC (i.v.)	AUC (conjugate) × 100/ AUC (naproxen p.o.)
Naproxen i.v.[c]	35	—	0.048	479	—	—
Naproxen p.o.[c]	19.7	2	0.049	439	91.6	—
Dex-T-10 conjugate p.o.	15.6	14	0.052	476	99.4	108.4
Dex-T-40 conjugate p.o.[d]	15.5	13	0.048	508	106.1	115.7
Dex-T-70 conjugate p.o.[c]	11.1	16	0.045	398	83.1	90.7
Dex-T-500 conjugate p.o.	11.6	18	0.044	422	88.1	96.1

[a] Each conjugate was administered to three pigs ranging in weight from 33 to 45 kg.
[b] Equal doses corresponding to 3.6 mg naproxen equivalents per body weight were given.
[c] From a previous study (8).
[d] A new group of three pigs was used.

characteristic of a good drug delivery system: (a) intact conjugate does not reach the systemic circulation, (b) the dextran backbone protects the attached drug from enzyme metabolism in the small intestine, (c) selective regeneration of the drug occurs in the cecum and the colon, and (d) bioavailability of naproxen from all the dextran prodrugs were close to 100%. The versatility of this approach to deliver other carboxylic acid agents as well as drugs containing other types of functional groups selectively to the colon is presently being investigated in this laboratory.

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