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Macromolecular prodrugs. XIV. Absorption characteristics of naproxen after oral administration of a dextran T-70-naproxen ester prodrug in pigs

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Summary

The bioavailability of naproxen after oral administration of aqueous solutions of a dextran T-70-naproxen ester prodrug in pigs was assessed. Compared to the administration of an oral solution of an equivalent dose of naproxen the average absorption fraction for the conjugate amounted to 91%. It was established that several features of the prodrug indicated that naproxen was released from the prodrug prior to systemic absorption and that drug activation involved the action of one or more enzyme systems located in the gastrointestinal tract. As was observed in rabbits, the plasma concentration–time curves for the conjugate were characterized by an initial lag time of about 2–3 h, whereas naproxen was detected in plasma immediately after p.o. administration of the drug compound, per se. The distribution of the prodrug along the GI tract at various times after conjugate administration was assessed qualitatively by HPLC analysis of the content of conjugated and free naproxen in various segments of the GI tract. From these experiments it was suggested that drug regeneration was effective in the bowel below the ileum. No visible allergic reactions appeared after oral administration of the dextran derivative to already i.v. sensitized pigs, indicating that the intact conjugate did not reach the systemic circulation.

Introduction

Generally, the bioavailability of a drug from a potential new oral drug delivery system is screened, using an animal model (Barr, 1972; Purich and Hunt, 1983). Due to the differences of the species in GI tract physiology, transit times of liquids and solids, and enzyme composition (Ings, 1984), the selection of a suitable animal model for testing a particular drug formulation may be difficult. A good correlation of drug bioavailability between humans and dogs has been reported for coumermycin A (Newmark et al., 1970), aminorex (Cressman and Sumner, 1971), amphetamine (Rosen et al., 1967) and penicillins (Poole et al., 1968; Poole, 1973). On the other hand, the dog model has provided less convincing correlations in case of flufenamic acid (Kaniwa et al., 1983), griseofulvin (Aoyagi et al., 1982) and diazepam (Ogata, 1982). Recently Dressman (1986) stated that the dog is of minor value for the assessment of oral sustained-

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release formulations. Rabbits have been employed successfully for GI drug absorption studies (Maeda et al., 1977, 1979; Takahashi et al., 1985; Aoyagi et al., 1984a). One disadvantage of the use of the rabbit model is the highly variable gastric emptying time of the animals (Maeda et al., 1980; Fischer and Riegelman, 1965; Chiou et al., 1969). The pig has been recommended for bioavailability tests for its similarity to humans in many physiological functions (Withey, 1973; Garcia et al., 1978). Comparable absorption characteristics of griseofulvin in the pig and man have been observed (Aoyagi et al., 1984b).

In a recent paper (Harboe et al., 1988a) we have reported on the bioavailability of naproxen after oral administration of dextran–naproxen ester prodrugs in rabbits. It was found that drug regeneration in the GI tract was facilitated by action of one or more enzyme systems. The present study was undertaken in order to determine naproxen absorption characteristics from orally administered dextran prodrugs, using the pig as an alternative animal model.

Materials and Methods

Naproxen ((+)-2-(6-methoxy-2-naphthyl)propionic acid) was obtained from Sigma, U.S.A. The dextran T-70 fraction (M_w 74000; M_n 36000) was purchased from Pharmacia, Sweden. The dextran–naproxen ester prodrug was synthesized as previously reported (Harboe et al., 1988b). Characterization of the dextran derivative was carried out according to our earlier studies (Johansen and Larsen, 1985; Larsen and Johansen, 1985). The degree of substitution (DS) has been expressed as the percentage of mg naproxen released per mg of the conjugate. In this study a prodrug derivative with DS of 8.2 was employed. Methanol used in the mobile phase was of chromatographic grade. All other chemicals were of analytical or reagent 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 chromato-integrator. The column size was 125 × 4.6 mm; it 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 (30–40 μm particles), Merck, F.R.G. Ultraviolet spectral and optical rotation measurements were performed with a Shimadzu UV-190 spectrophotometer, using 1 cm quartz cells, and a Perkin Elmer Model 141 polarimeter, respectively. Readings of pH were done with a Radiometer Type pHM 26 meter at the temperature of study.

Bioavailability studies

Three female pigs (Danish land race/Yorkshire) from a SPF production herd, weighing 41–45 kg were used in this study. They were housed in individual pens, kept at a 12:12-h light/dark cycle at 22° C. The pigs were fed twice a day with a standard pelleted diet and provided with water ad libitum.

The pigs had inserted sterile polyurethane catheters of 1.4 mm internal and 2.1 mm external diameter in the external jugular vein for blood sampling. Surgery was performed under aseptic conditions under general anesthesia. The vein was exposed through a 5 cm incision parallel to and slightly lateral of the ventral midline of the neck. Prior to the insertion, the catheter was passed s.c. from the skin incision to a position dorsal to the neck. The vein was ligated and the catheter inserted 20–25 cm just central to the ligature and fixed by a silk ligature. The catheter was closed with a stopper with a latex membrane and flushed with heparinized saline and protected by an elastic adhesive bandage round the neck of the pig.

The animals were fasted overnight prior to peroral drug administration, while water was allowed ad libitum. An aqueous solution of the dextran conjugate (corresponding to 1 mg free naproxen per ml) was given mixed with food. The animals were fed in connection with each blood sampling. A solution of parent naproxen (0.5 mg/ml) with pH 7.5 was administered likewise. The i.v. solution of naproxen (20 mg/ml) with pH 9.6 was filtered through a 0.22 μm filter before it was injected. All doses administered corresponded to 3.6 mg naproxen per kg body weight.

Analytical procedure

Approximately 5 ml of an isotonic saline solution containing heparin (50 I.E./ml) was injected into the catheter followed by a withdrawal of 5 ml blood. With another syringe a 2 ml blood sample was taken and after centrifugation the plasma was frozen at −20 °C and the former 5 ml of blood was reinjected. The catheter was finally washed with 5 ml of heparinized saline.

After thawing, a 100 µl plasma sample was deproteinized through the addition of 300 µl of methanol. The mixture was vortexed and centrifuged at 10000 *g* for 3 min. The supernatant was prepared for HPLC analysis by using an autosampler. The mobile phase consisted of methanol: 0.02 M phosphate buffer pH 2.5 (65 : 35 v/v). The flow rate was maintained at 1 ml/min and the column effluent was monitored at 271 nm. Naproxen plasma samples were treated likewise and were used as external standards. Standard samples were run after every sixth blood sample. In separate experiments it was established that naproxen was stable in the precipitation media for at least 24 h as was evidenced by HPLC analysis.

Results and Discussion

The 3 pigs were each orally given solutions of the dextran T-70-naproxen ester prodrug and naproxen. Due to a clogged catheter data for i.v. administered naproxen were only obtained from two of the animals. During the 4 days between the experiments, the animals were at rest and fed a standard diet.

Fig. 1A illustrates the average plasma profile following i.v. administration of parent naproxen in two pigs. The shapes of the individual profiles were quite similar, the absolute values, however, of the naproxen plasma concentration varied considerably. From a semilogarithmic plot of the plasma concentration vs time data (Fig. 1B) it is apparent that the animals showed biexponential elimination of naproxen from the systemic circulation after i.v. administration of the drug. Generally, curves of this shape are fitted to an equation of the form $C_p = A\ e^{-\alpha t} + B\ e^{-\beta t}$ derived from the two-compartment open-model system. However, in the treatment of the plasma data, the initial distributional phase was ignored and the simple

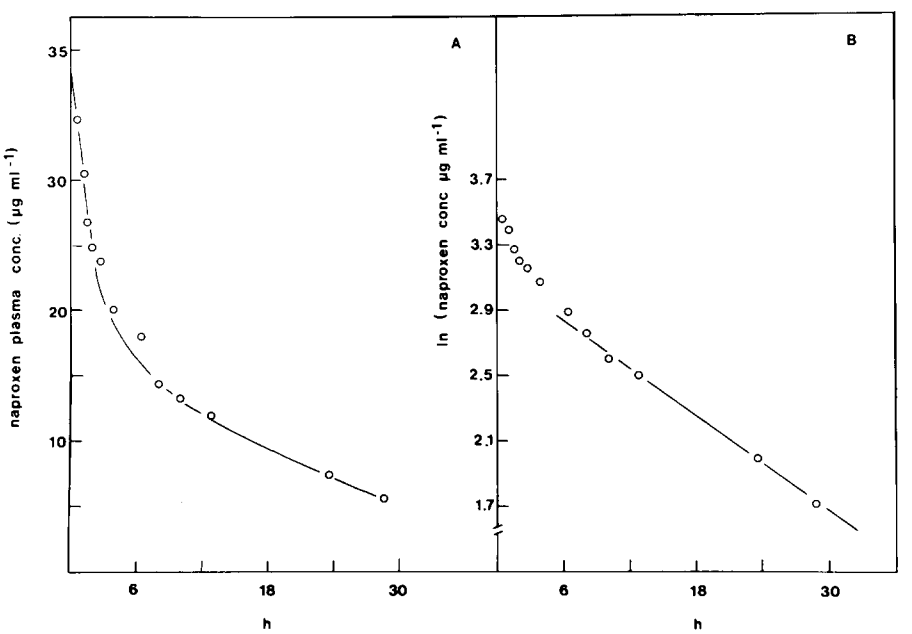

Fig. 1. A: average naproxen plasma concentration–time curve from 2 pigs (49–51 kg) after i.v. administration of a naproxen solution (3.6 mg per kg body weight). B: semilogarithmic plot of naproxen plasma concentration vs time.

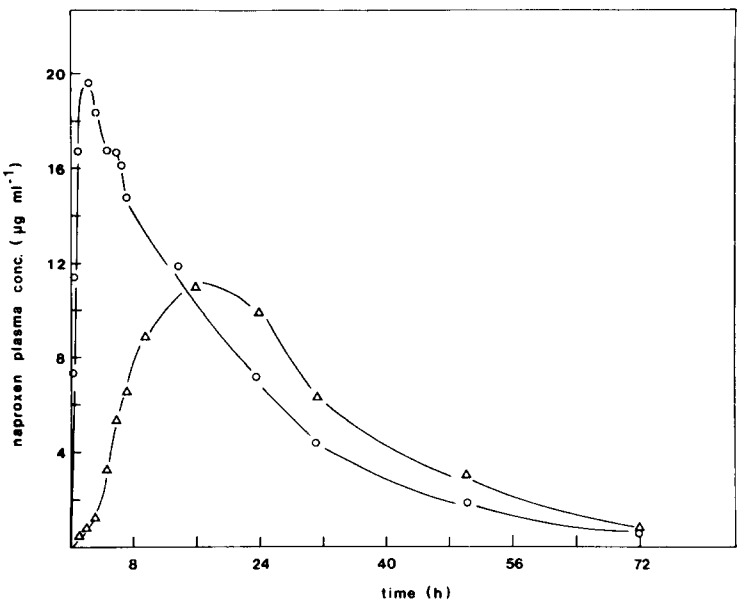

Fig. 2. Average naproxen plasma concentration-time curves from 3 pigs (40–44 kg) after oral administration of an aqueous solution of naproxen (3.6 mg/kg b. wt.) (○) and an equivalent dose of a dextran T-70 conjugate (DS 8.2) (△).

one-compartment open model was used in the calculations. Thus the area under the curve was obtained from the equation:

$$AUC_{0-\infty} = C_0/\beta \quad (1)$$

where β represents the apparent terminal elimination rate constant and C_0 is the initial plasma concentration as determined from the intercept of a semilogarithmic plot of peak area against time. The pharmacokinetic parameters calculated on this basis are listed in Table 3. The half-life of the terminal phase (14.3 h) was close to those in humans after i.v. injection (13.9 h, Runkel et al., 1972; Runkel et al., 1973) and p.o. administration (17 h, Maggi and Renzetti, 1988). Surprisingly, identical experiments carried out using minipigs (breed not specified) revealed a naproxen β half-life of 4.8 h (Runkel et al., 1972).

After oral administration the AUC was derived, employing the equation:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/\beta \quad (2)$$

where β was determined by log-linear regression of the terminal part of the curve. C_t is the plasma

TABLE 1

Pharmacokinetic parameters of naproxen after oral administration of a solution of the drug, equivalent to 3.6 mg free naproxen per kg body weight, in pigs

Parameter	Pig		
	A (40 kg)	B (42 kg)	C (43.5 kg)
Plasma conc. (μg/ml)			
0.2 (h)	8.845	9.210	4.090
0.5 (h)	14.929	12.648	6.340
0.9 (h)	20.025	15.831	14.510
2.2 (h)	19.739	18.404	20.934
2.9 (h)	17.484	18.107	19.814
4.6 (h)	15.530	15.628	18.980
5.3 (h)	16.123	14.585	19.561
6.3 (h)	14.692	14.678	19.143
7.1 (h)	14.092	13.406	16.695
13.4 (h)	10.701	11.103	13.680
23.4 (h)	6.776	7.276	7.282
31.3 (h)	3.718	4.750	4.756
50.3 (h)	1.554	2.301	1.761
72.0 (h)	0.553	0.917	0.442
T_{max} (h)	0.9	2.2	2.2
C_{max} (μg/ml)	20.0	18.4	20.9
AUC (μg/ml·h)	407.3	450.5	458.6

TABLE 2

Pharmacokinetic parameters of naproxen after oral administration of a dextran T-70-naproxen ester prodrug, equivalent to 3.6 mg free naproxen per kg body weight, in pigs

Parameter	Pig		
	A (44 kg)	B (41 kg)	C (44.5 kg)
Plasma conc. (μg/ml)			
1.0 (h)	0.417	0.614	0.749
2.0 (h)	0.464	0.896	0.982
3.0 (h)	1.001	1.391	1.552
4.6 (h)	3.420	3.333	2.751
5.8 (h)	5.450	5.151	4.245
7.0 (h)	7.624	5.812	6.216
9.6 (h)	10.176	7.488	9.007
15.6 (h)	13.012	9.087	10.830
23.7 (h)	11.559	8.118	9.646
31.0 (h)	7.456	5.582	6.007
50.5 (h)	3.675	2.680	2.673
71.9 (h)	1.092	1.141	0.986
T_{max} (h)	15.6	15.6	15.6
C_{max} (μg/ml)	13.0	9.0	10.8
AUC (μg/ml·h)	456.9	355.0	381.9

concentration at time t. AUC_{0-t} was calculated by using the trapezoidal rule. In Table 1 are summarized the pharmacokinetic parameters obtained after p.o. administration of a solution of parent naproxen (pH 7.5) in the 3 pigs. In comparison to i.v. administration, the interindividual variation of the latter data was less pronounced. The average naproxen plasma concentration vs time curve is depicted in Fig. 2. Although naproxen is expected to precipitate in the acidic environment of the gastric juice, the drug appears rapidly in the blood with a T_{max} value ranging from 0.9 to 2.2 h.

The almost identical elimination rate constants obtained after oral and i.v. administration might also reflect a rapid absorption of the drug from the GI tract. The individual absorption data obtained after oral administration of the dextran–naproxen ester prodrug are presented in Table 2 and Fig. 3, revealing fairly good correlation of the pharmacokinetic parameters between the animals. As seen from Fig. 2, the time dependence of the naproxen appearance in the systemic circulation after p.o. intake of the parent drug and the dextran prodrug, respectively, differs significantly. The prodrug provides a more gradual increase in drug plasma concentration in proportion to the one observed after p.o. administration of the free drug entity. The enhancement of T_{max} by a factor of 8 is accompanied by a 2-fold reduction in the mean

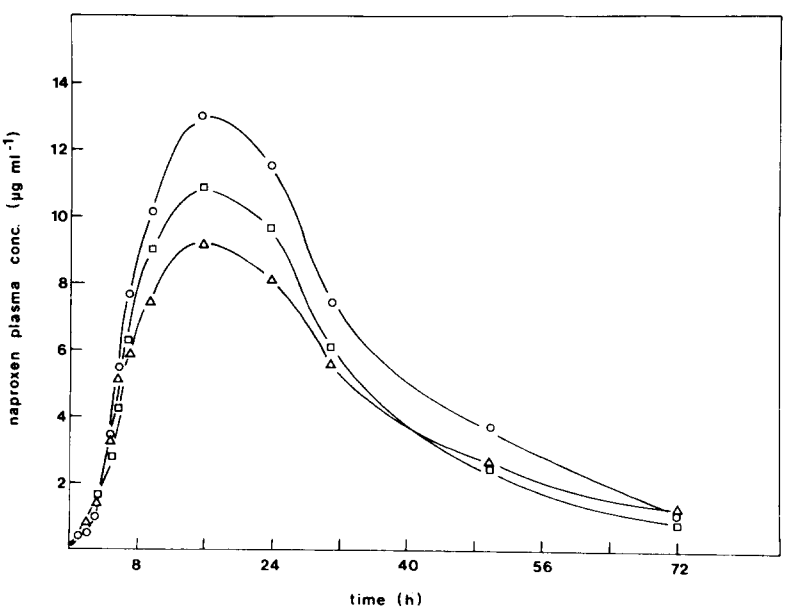

Fig. 3. Naproxen plasma concentration–time profiles after oral administration of a solution of a dextran T-70 prodrug (DS 8.2) corresponding to 3.6 mg naproxen per kg in pigs. (○), 44 kg; (△), 41 kg; (□), 44.5 kg.

TABLE 3

Bioavailability of naproxen after oral administration of a dextran T-70-naproxen ester prodrug in pigs

Compounds	C_{max} (μg/ml)	T_{max} (h)	β (h^{-1})	AUC (μg/ml · h)	AUC (p.o.) / AUC (i.v.) (%)	AUC (conjugate) / AUC (naproxen p.o.) (%)
Naproxen i.v.	~ 35	–	0.0483	479 *	–	–
Naproxen p.o.	19.7	2.2	0.0489	439	91.6	–
Dextran T-70 conjugate p.o.	11.1	16	0.0447	398	83.1	90.7

Average pharmacokinetic data from 3 pigs, determined after administering a solution of the prodrug, in comparison with those obtained after p.o. and i.v. administration of solutions of parent naproxen. All doses were equivalent to 3.6 mg free naproxen per kg body weight.

* Average of two pigs.

plasma level. From the summarized absorption characteristics in Table 3, the relative bioavailability of naproxen from the dextran prodrug amounted to approximately 90%. Similar experiments carried out using the rabbit as an animal model revealed a bioavailability of 62% (Harboe et al., 1988a). This difference in absorption fractions might be a reflection of the variation of GI transit times or enzyme composition between the two species. The plasma elimination rate constant calculated after prodrug administration is somewhat smaller than the value found after administering the free drug. However, the explanation for this difference could be that the prodrug's naproxen absorption phase is not totally completed, even after 40 h.

Several features of the dextran prodrug are indicative of naproxen being released from the conjugate in the gastrointestinal tract of the pig prior to systemic absorption. First, extensive absorption of the intact macromolecular prodrug from the GI-tract is not to be expected (O'Hagan et al., 1987; Ekström et al., 1988). Secondly, the regeneration of naproxen from the prodrug in pig plasma and liver homogenate (with half-lives of 172 and 160 h, respectively (Larsen and Johansen, 1989)) proceeds too slowly to afford the actual naproxen plasma profile. Thirdly, no allergic reactions were observed after oral administration of the prodrug to already i.v. sensitized animals. As discussed previously, using the rabbit as the animal model (Harboe et al., 1988a) pure hydrolytic release of naproxen from the dextran prodrugs cannot account for the initial phase of systemic drug absorption, and thus drug liberation apparently requires the involvement of one or more enzyme systems located within the GI tract.

The absorption curve obtained for the dextran conjugate in the pig exhibits an initial lag time of drug absorption of approximately 2–3 h. Similarly, the plasma concentration vs time profiles, after administering orally dextran–naproxen ester prodrugs in rabbits, were characterized by a delayed appearance of naproxen in plasma. Based on preliminary experiments, using rabbit homogenates of various parts of the GI-tract, it was suggested that drug activation occurred primarily in the caecum and the colon. Information is lacking in the literature concerning the gastrointestinal transit times of liquids and solids in the pig. With reference to other species, including man (Davis, 1985), it is assumed that the dextran prodrug in solute state arrives to the small intestine independently of the stomach emptying time of the pig. To get a qualitative picture of the distribution of the dextran prodrugs along the gastrointestinal tract at various times after conjugate administration, 3 pigs were given the derivative orally, mixed in the food. The animals were killed after 2, 4 and 6 h, and representative segments of the GI tract were excised. The contents of the various segments were removed and lyophilized. The total amount of naproxen was determined by HPLC after base treatment of the samples. The presence of free naproxen in the GI tract segments was obtained, likewise omitting base addition to the samples.

TABLE 4

Relative amounts of conjugated and free naproxen found in various segments of the GI tract of pigs at different times after oral administration of solutions of a dextran T-70-naproxen ester prodrug

	2 h		4 h		6 h	
	conjugated	free	conjugated	free	conjugated	free
Stomach	10	1	–	–	–	–
Duodenum	–	–	–	–	–	–
Jejunum	1	–	–	–	–	–
Ileum	18	–	–	–	–	–
Caecum	3	–	19	6	1	1
Colon I	–	–	6	3	3	1
Colon II	–	–	–	–	1	–
Rectum	–	–	–	–	–	–

The amount of naproxen covalently attached to dextran was calculated as the difference between the total amount and free naproxen. The analytical data are presented in Table 4.

After 2 h a considerable amount of the administered prodrug dose is still residing in the stomach, whereas at later sampling times, the conjugate as well as free naproxen were only observed in the caecum and the colon. Comparison of these data with the naproxen absorption profile indicates that naproxen regeneration takes place in the GI tract below the ileum. In homogenate experiments we have recently substantiated this suggestion (to be published elsewhere).

It is a well-established fact that orally administered macromolecules like gamma globulins pass readily into the circulation in the newborn of various species (Halliday, 1955; Kraehenbuhl and Campiche, 1969) including man (Leissring et al., 1962; Morris, 1968). Although the adult mammalian gut is regarded as largely impermeable to macromolecular compounds, animal studies have shown that small amounts of proteins can cross the mucosal barrier (Warshaw et al., 1971; Warshaw et al., 1974; Ducroc et al., 1983). The macromolecular transport efficiency in man varies between the various parts of the GI tract (Fordtran et al., 1965; Sundqvist et al., 1980; Loehry et al., 1973) with the lymph system constituting the predominant uptake route (Katayama and Fujita, 1972; Walker et al., 1972; Caldwell et al., 1982). Due to the hydrophilic character of the dextran molecule the absorption potential of such compounds is presumably negligible. However, attachment of the lipophilic agent naproxen to the polymer may alter the hydrophilic-lipophilic balance of the prodrug in a direction favouring lymphatic uptake (Charman and Stella, 1986; Palin et al., 1982; Takada et al., 1985). The possible eliciting capacity of the dextran prodrugs after p.o. administration was studied as follows. In connection with the surgical insertion of the catheter, the pigs were given about 300 mg iron dextran i.m. In the following 4-week period, the animals were successively administered orally dextran–naproxen ester prodrugs of molecular weights of 10 000, 70 000 and 500 000. The pigs showed no visible discomfort from this repeated administration. After this treatment one pig was injected i.v. a 100 mg dose of a dextran T-500-naproxen conjugate. Almost instantly, an anaphylactoid reaction developed and the animal was unconscious for 2 min. After this period, the pig got up and started to eat again. The same happened to a second pig, which received a much smaller dose of the T-500 conjugate. A third pig, which was not treated with iron dextran, did not have any reaction after i.v. administration of 100 mg of the latter derivative. The iron dextran preparations contain dextran fractions of much higher molecular weight than those used clinically as plasma expanders, and in man manifestations of anaphylactoid reactions have been observed after repeated administration of such iron products (Hamstra et al., 1980). It is therefore likely that iron dextran treatment results in sensitization of the animals. After a 4-week

period reinjection of even a very small amount of a dextran T-500 conjugate was sufficient to provoke an allergic reaction. No visible reaction appeared after oral administration of the dextran prodrugs suggesting that allergenic conjugate fractions are not absorbed from the gastrointestinal tract.

In the present study it was found that the bioavailability of naproxen in the pig after oral administration of a dextran T-70-naproxen ester prodrug was close to 100% compared to an equivalent oral dose of the parent drug, and that drug activation took place in the lower part of the GI tract. In a series of experiments (to be published elsewhere) we have recently established that drug regeneration occurs selectively in the caecum and the colon of pigs.

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