Multiple Oral Administration of a Ketoprofen–Dextran Ester Prodrug in Pigs: Assessment of Gastrointestinal Bioavailability by Deconvolution

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Deconvolution has been applied to estimate the *in vivo* dissolution/release process of ketoprofen from a ketoprofen–dextran ester prodrug in pigs. The prodrug was given to three pigs at intervals of 12 hr and in seven doses corresponding to 4 mg ketoprofen/kg body weight. Frequent blood sampling was carried out at the first, third, and seventh intervals. Plasma steady-state concentrations of ketoprofen following the prodrug administration were between 2 and 4 μg/ml. The reference consisted of a single p.o. dose of parent ketoprofen (4 mg/kg body weight). For each pig the response following the multiple dosing was deconvolved with the reference response using an algebraic deconvolution procedure adopted from the literature. The obtained cumulated *in vivo* dissolution/release profiles revealed similar release rates for the three pigs and similar extents of release (59, 70, and 65%). The mean *in vivo* dissolution/release times (MDT) were calculated to be 5.4, 6.1, and 5.7 hr, respectively. In conclusion, following administration of the dextran prodrug the plasma concentration curves and the dissolution/release profiles are uniform, with small interindividual variations.

KEY WORDS: ketoprofen; prodrugs; pharmacokinetics; deconvolution; *in vivo* dissolution/release profile.

INTRODUCTION

After p.o. administration of naproxen–dextran ester prodrugs to pigs, the bioavailability of the NSAID compound was found to be nearly complete (1,2). Naproxen was attached to the dextran carrier molecule in the form of a biolabile ester bond. Further, the drug was released from the prodrugs enzymatically and exclusively in the cecum and the colon of the pigs (3). Hence, dextran prodrugs can provide colon site-specific delivery of antiinflammatory agents. Similar results were obtained with ketoprofen–dextran ester prodrugs (4). Further, the plasma concentration–time profiles indicated that such dextran prodrugs possess considerable sustained release capacity.

The aim of the present study was to investigate the steady-state plasma concentration–time profiles of ketoprofen administering the prodrug in a multiple-dosing regimen. Deconvolution has become an important tool in the assessment of *in vivo* dissolution/release and absorption (e.g., Refs. 5–7). Therefore, for detailed analysis of the *in vivo* dissolution/release profiles of ketoprofen, an algebraic deconvolution technique was applied.

MATERIALS AND METHODS

Animals. Three female pigs (Danish land race/Yorkshire, weighing from 36 to 39 kg) from a SPF production herd were used. They were housed in individual pens, kept under 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 done as previously described (1).

Test Procedure. The pigs were fasted overnight prior to the drug administration, while water was allowed *ad libitum*. An aqueous solution of the ketoprofen–dextran ester prodrug (equivalent to 1 mg ketoprofen/ml) was mixed with the food and given in a dose corresponding to 4 mg ketoprofen/kg body weight. The administration was repeated six times at a dosing interval of 12 hr. On the basis of the plasma concentration–time profile from a single dose of the prodrug in a pig from an earlier study (4), a sampling scheme was designed. Blood sampling (a total of 40 samples) was carried out, with frequent sampling at the first, third, and seventh dose intervals and two or three samples in the remaining intervals in order to measure the trough concentrations.

In a similar manner a solution of parent ketoprofen (1 mg/ml) was administered as a single dose of 4 mg/kg body weight. The ketoprofen–prodrug and ketoprofen were administered in the order mentioned, with a washout period of 4 days.

Drugs. Ketoprofen [2-(3-benzoylphenyl)propionic acid] was obtained from Sigma (St. Louis, MO). The dextran fraction T-70 (M_w, 72,200; M_n, 38,400) was purchased from Pharmacia (Uppsala, Sweden). The ketoprofen–dextran ester prodrug was synthesized as reported previously (8) and characterized according to earlier studies (9,10).

Analytical Procedure. Five hundred microliters of plasma was deproteinized with 1500 μl of methanol. The mixture was vortexed and centrifuged at 10,000*g* for 5 min. The supernatant was prepared for HPLC analysis 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 1 ml/min and the effluent was monitored at 260 nm. Ketoprofen was quantified by peak-area measurements using ketoprofen as external standard.

The Hitachi chromatographic system consisted of a L-6200 Intelligent pump, a L-4000 variable-wavelength detector, a 655A-40 autosampler, a D2000 chromatointegrator, and a Rheodyne Model 7125 injection valve with a 20-μl loop. The column (125 × 4 mm) was packed with Spherisorb ODS-1 (5 μm; Phase Sep, UK) and connected with a small precolumn packed with Perisorb RP-8 (30–40 μm; Merck, FRG). Methanol used in the mobile phase was of chromato-

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graphic grade. All other chemicals were of analytical or reagent grade.

Pharmacokinetic Analysis. Deconvolution has become an important tool for the assessment of the *in vivo* drug input rate, which comprises dissolution/release and absorption (e.g., Refs. 5–7).

If a linear relationship exists between the rate of drug release in the gastrointestinal tract, $f(t)$, and the resulting response, i.e., the plasma concentration level, $c(t)$, this relationship can be expressed as a convolution integral:

$$c(t) = c_\delta(t) * f(t) = \int_0^t c_\delta(u) \cdot f(t-u)du \qquad (1)$$

In this case $c(t)$ and $c_\delta(t)$ represent the plasma concentrations of ketoprofen following administration of the ketoprofen–dextran ester prodrug and parent drug, respectively. By deconvolving $c(t)$ with $c_\delta(t)$, an *in vivo* dissolution/release profile of ketoprofen from the prodrug can be determined.

In a previous study (in three similar pigs) the linearity was confirmed with respect to the plasma concentrations of ketoprofen following administration of the prodrug at doses of 2, 4, and 8 mg/kg body weight (4).

There are several numerical deconvolution algorithms which allow the use of raw data directly as input (e.g., Refs. 11–13). However, this requires equally spaced sampling intervals for $c(t)$ and $c_\delta(t)$. If this is not the case, an interpolation procedure is necessary. In the present study an algebraic deconvolution algorithm was chosen to which the input consists of parameters of polyexponential expressions which describe $c(t)$ and $c_\delta(t)$, respectively (5).

An algebraic deconvolution algorithm by Gillespie and Veng-Pedersen (5) was adopted from the literature. The algorithm (DCON) was rewritten in TURBO-PASCAL vs. 5.0. The polynomial root-finding subroutine (ZPOLR) was replaced by a Laguerre procedure from the numerical toolbox of TURBO-PASCAL. The program was evaluated using the data from the original publication (5).

The input to the algorithm consists of parameters in polyexponential expressions. Equation (2) was fitted to the plasma concentrations of ketoprofen following administration of ketoprofen:

$$c_k(t) = D_k \cdot c_\delta(t) = \sum_{i=1}^{n} a_i \cdot e^{-\alpha_i(t-t_{01})}, \qquad t \geq t_{01} \qquad (2)$$

where $n = 2$ or 3, D_k is the dose, and t_{01} is the lag time.

The ketoprofen plasma concentrations following multiple dosing of the ketoprofen–dextran ester prodrug were approximated by Eq. (3):

$$c(t) = \sum_{i=1}^{m} b_i \cdot \frac{1 - e^{-z\beta_i\tau}}{1 - e^{-\beta_i\tau}} \cdot e^{-\beta_i(t-t_{02})}, \qquad t \geq t_{02} \qquad (3)$$

where $m = 3$, z is the number of doses administered (1–7), τ is the dosing interval (12 hr), t is the time in dose interval ($0 \leq t \leq \tau$), and t_{02} is the lag time.

The analytical deconvolution of Eq. (1) yields an expression of the rate of input $f(t)$, which on integration and multiplication by $100/D$, yields an expression for the cumulative percentage of the dose D released at time t, $PCT(t)$:

$$PCT(t) = u_0 + \sum_{i=1}^{m+n-2} u_i \cdot e^{-v_i(t-t_0)}, \; t \geq t_0, \qquad t_0 = t_{02} - t_{01} \qquad (4)$$

where u_0 and the u_i and v_i parameters are output from the deconvolution algorithm. From these parameters it is possible to estimate the mean *in vivo* dissolution/release time MDT (5,15).

In Eq. (4) it is assumed that $f(t)$ after administration of dextran-linked ketoprofen is adequately described by a sum of exponentials.

For a derivation of the general expression of this analytical deconvolution cf. Ref. 14.

Curve fitting of Eqs. (2) and (3) to the plasma concentration–time data were carried out using the simplex algorithm (16) in a modified version of a curve-fitting program adopted from the literature (17). Nonweighting and weighting factors of $1/c$ or $1/c^2$ were applied as appropriate. The number of exponential terms (n and m) was determined from the goodness of fit, which was evaluated from the residual sum of squares, the Akaike information criterion [AIC (19)], and plots of the residuals.

RESULTS

The plasma concentration–time profiles of ketoprofen following administration of seven doses of the ketoprofen–dextran ester prodrug (each corresponding to 4 mg ketoprofen/kg body weight) are shown in Fig. 1. In Fig. 1b, the data points marked with asterisks (in the first dosing interval) were not included in the curve fitting due to difficulties in obtaining blood during this period. The parameters obtained from the curve fitting are listed in Table I. The lag times (t_{02}) were estimated to be 1.3, 1.8, and 1.7 hr. Similar values have been observed for dextran ester prodrugs in earlier studies [ketoprofen (4), naproxen (1–3)].

Figure 2 shows the corresponding profiles following administration of a single dose of parent ketoprofen (4 mg/kg; fitted parameters in Table I). The curves without data points represent simulated profiles of a single dose of the prodrug calculated according to the b and β parameters in Table I.

The deconvolution results are also shown in Table I and profiles of the cumulated percentage released ketoprofen from the prodrug are depicted in Fig. 3. The cumulated percentages drug released, estimated as u_0 (i.e., at time infinity), were calculated to 58.9, 70.2, and 64.8%, respectively. From the u and v parameters in Eq. (5) (or visual inspection of the profiles in Fig. 3), it is possible to estimate the time for 50% of the dose release ($t_{50\%}$). These values were estimated to be 8.7, 8.1, and 7.8 hr (calculated from the time of administration). Further, the times from the dose administered to the release of 50% of the bioavailable amount [$PCT(t_{50\%}b) = U_0/2$] were 4.7, 5.9, and 5.0 hr, and, MDT

$$\left[\text{MDT} = t_0 - \frac{1}{U_0} \cdot \sum_{i=1}^{m+n-2} \frac{U_i}{V_i}\right]$$

was 5.4, 6.1, and 5.7 hr.

Using the trapezoidal rule, the relative amount of drug

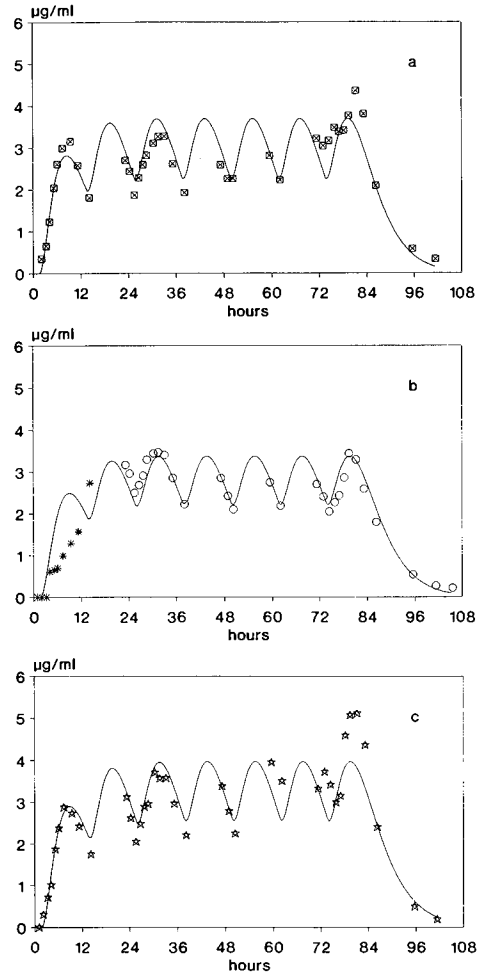

Fig. 1. Plasma concentration profiles of ketoprofen in three pigs (a–c) following administration of seven doses of a ketoprofen–dextran ester prodrug (equivalent to 4 mg/kg ketoprofen). Curves represent the fitted approximation of the response. For pig b the data points marked with asterisks were not included in the curve fitting.

Table I. Polyexponential Parameters Obtained Fitting to the Observed Plasma Concentration–Time Data Following Administration of Ketoprofen (the a and α Parameters) and of Ketoprofen–Dextran Ester Prodrug (the b and β Parameters)[a]

	Pig a	Pig b	Pig c
Ketoprofen			
a_1 (mg/L)	14.7	6.1	12.1
a_2 (mg/L)	−14.7	39.7	−12.1
a_3 (mg/L)	—	−45.8	—
α_1 (hr^{-1})	0.179	0.141	0.159
α_2 (hr^{-1})	0.801	0.631	0.903
α_3 (hr^{-1})	—	0.807	—
n	2	3	2
D_k (mg/kg)	4	4	4
t_{01} (hr)	0	0.05	0
Ketoprofen–dextran			
b_1 (mg/L)	119.1	82.5	29.6
b_2 (mg/L)	−208.8	−171.6	−400.0
b_3 (mg/L)	89.7	89.1	370.4
β_1 (hr^{-1})	0.227	0.212	0.177
β_2 (hr^{-1})	0.284	0.277	0.342
β_3 (hr^{-1})	0.362	0.337	0.355
m	3	3	3
D (mg/kg)	4	4	4
t_{02} (hr)	1.29	1.77	1.74
Deconvolution results			
u_0 (%)	58.87	70.16	64.79
u_1 (%)	160.50	566.2	24.72
u_2 (%)	−441.21	−3214.5	−1338.0
u_3 (%)	219.83	6861.0	1248.7
u_4 (%)	—	−4282.3	—
v_1 (hr^{-1})	0.227	0.212	0.177
v_2 (hr^{-1})	0.284	0.277	0.342
v_3 (hr^{-1})	0.362	0.322	0.355
v_4 (hr^{-1})	—	0.337	—
$t_{50\%}b$ (hr)	4.70	5.88	5.01
$t_{50\%}$ (hr)	8.71	8.12	7.80
MDT (hr)	5.35	6.13	5.69

[a] The u and v parameters are outputs from the deconvolution procedure. $t_{50\%}$ corresponds to the time from administration for release of 50% of the administered dose. Similarly, $t_{50\%}b$ equals the time from administration for the release of 50% of the bioavailable amount (u_0). MDT is the mean *in vivo* dissolution (or release) time.

release, F_{rel}, in the third and the seventh dosing intervals was estimated to be 49.0, 73.4, and 55.9 and 62.5, 63.1, and 74.0%, respectively. The average F_{rel} for the third and the seventh intervals was 55.7, 68.2, and 65.0% for the three pigs, respectively.

DISCUSSION

Multiple dosing of the ketoprofen–dextran ester prodrug in the three pigs resulted in reasonably uniform plasma concentration–time profiles of ketoprofen (Fig. 1). The steady-state plasma concentrations estimated from the fitted profiles were between 2 and 4 μg/ml, with fluctuations of less than ±1 μg/ml. Steady state was reached in the second dosing interval. Taking into account the twice-a-day dosage regimen and the calculated plasma elimination half-life of ketoprofen of 4 hr, the results demonstrate that the dextran prodrugs exhibit considerable sustained release capacity. In pigs a and c (Figs. 1a and c), there was a trend toward an increase in the plasma concentrations in the seventh dosing interval, which was not investigated further.

The *in vivo* dissolution/release profiles shown in Fig. 3 exhibit similar time courses, indicating small interindividual variations in ketoprofen release, averaging 65%. In comparison, earlier observations from a single dose of the prodrug in pigs (4) resulted in an average of 72%. No information on the absolute availability of ketoprofen can be obtained from this study, but earlier findings indicate complete absorption (4). Further, the rate of dissolution/release calculated as the time for 50% of the dose to be released was similar in the three pigs.

The mean dissolution (or release)-time MDT can also be used to describe the overall dynamics of drug release. In this case "mean" refers to the time when 50–60% of the available amount of drug has been released [PCT(MDT)/u_0 · 100%].

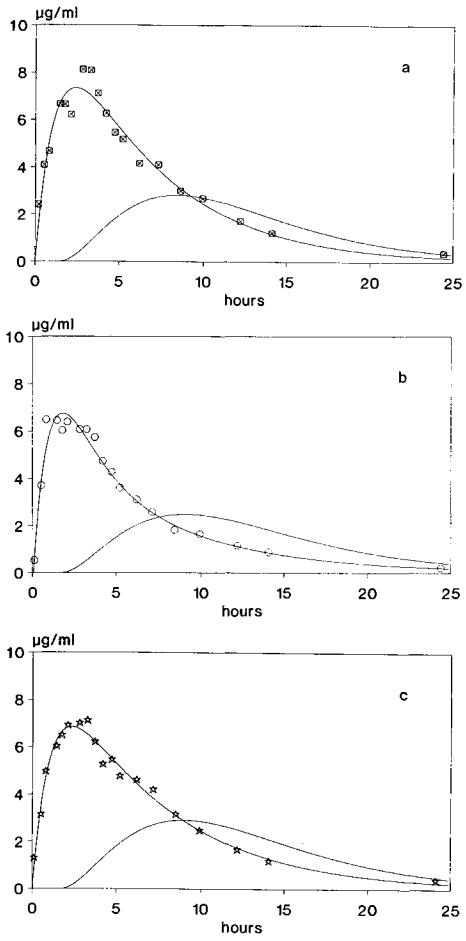

Fig. 2. Fitted plasma concentration profiles of ketoprofen in three pigs (a–c) following administration of a single dose of 4 mg/kg ketoprofen. The curves without data points represent the plasma concentration profile of the ketoprofen–dextran ester prodrug following a single dose (equivalent to 4 mg/kg ketoprofen). These curves are simulated using the parameters obtained from the multiple-dosing plasma concentration profile (cf. Fig. 1 and Table I).

As expected, this value is between the theoretical value for zero-order (50%) and that for first-order (63%) kinetics (15). The MDT may be used for *in vitro–in vivo* correlation studies (e.g., Ref. 18), and it is readily available from the output

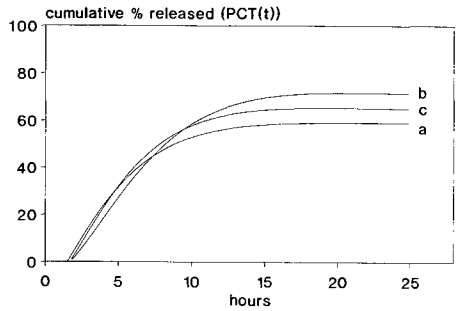

Fig. 3. Cumulated *in vivo* release profiles of ketoprofen from a ketoprofen–dextran ester prodrug in three pigs (a–c). The curves are obtained by deconvolution of the plasma concentration profile from the prodrug administration [$c(t)$] with the profile from the ketoprofen administration [$c_\delta(t)$; cf. Fig. 2].

parameters of the deconvolution algorithm. As steady state has already been reached in the second dosing interval, the sampling strategy in this multiple-dosing study makes it possible to apply the usual AUC approach (area under the curve over 12 hr) in estimating the relative bioavailability, F_{rel}. The apparent increase in the plasma concentrations in pigs a and c is reflected, as F_{rel} increased by a factor of 1.3, whereas in pig b it decreased by a factor 0.9. However, there is a close agreement between the u_0 values and the average values of F_{rel} (differences between 0.3 and 5%).

In conclusion, the estimated *in vivo* dissolution/release profiles and their related parameters indicate small interindividual variations. Earlier studies of naproxen–dextran ester prodrugs in pigs revealed similar sustained-release properties as judged from the single-dose study (1,2). This study shows that multiple dosing of the dextran prodrug in pigs seems to result in reproducible plasma concentration–time courses.

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