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Dextran-methylprednisolone succinate as a prodrug of methylprednisolone: dose-dependent pharmacokinetics in rats

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Abstract

The dose-dependency in the pharmacokinetics of a macromolecular prodrug of methylprednisolone (MP), dextran-methylprednisolone succinate (DMP), was investigated in rats. Single doses (MP equivalent) of 2.5, 5.0, 10, 20, and 30 mg/kg of DMP were administered intravenously to rats ($n = 5$/group), and serial blood samples (0–96 h) and spleen and liver tissues (96 h) were collected. The concentrations of DMP in plasma and spleen were analyzed using a size-exclusion chromatographic method. The concentrations of DMP in the liver samples were determined by an indirect method after sequential hydrolysis by dextranase and esterase enzymes, followed by HPLC analysis of MP. The kinetics of DMP were analyzed by non-compartmental methods. The systemic clearance of DMP decreased $\approx$5-fold (from 42.1 ± 11.0 to 7.72 ± 1.84 ml/h per kg) when the dose was increased from 2.5 to 30 mg/kg. The nonlinearity in the clearance of DMP could be adequately described by a Michaelis–Menten type elimination with a maximum velocity of elimination of 1.72 mg/h per kg and a constant of 24.9 μg/ml. Additionally, the percent of the dose of DMP found at 96 h in the liver and spleen, where the prodrug is sequestered and gradually eliminated, significantly decreased with an increase in the dose. It is concluded that the clearance of DMP in rats is modestly dose-dependent in the dosage range of 2.5–30 mg/kg.

Keywords: Dextran-methylprednisolone conjugate; Prodrug; Nonlinear pharmacokinetics; Nonlinear clearance; Liver uptake

1. Introduction

The glucose polymers dextrans have been investigated as macromolecular vehicles for targeted and sustained delivery of different therapeutic and imaging agents, including anticancer drugs (Larsen, 1989; Takakura and Hashida, 1995; Mehvar, 2000a). We have shown (Mehvar et al., 1994) that after systemic administration, dextrans with high molecular weight ($\sim$70 000) substan-

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tially accumulate in the reticuloendothelial organs, such as liver and spleen. It was suggested (Mehvar et al., 1994; Mehvar, 2000a), therefore, that dextrans of high M_w may be suitable for targeting drugs to these organs.

Conjugates of dextran with glucocorticoids were first synthesized by McLeod et al. (1993, 1994) for the local delivery of steroids to the colon after oral administration. We used the same approach for systemic delivery of methylprednisolone (MP) as an immunosuppressant to the reticuloendothelial system. A conjugate of MP with 70 000 dextran (DMP) was synthesized (Mehvar, 1999) using succinic acid as a linker, and the hydrolysis kinetics (Mehvar, 1999; Mehvar et al., 2000a), pharmacokinetics (Zhang and Mehvar, in press) and pharmacodynamics (Mehvar and Hoganson, 2000; Rensberger et al., 2000) of the conjugate were investigated in rats. It was shown (Mehvar and Hoganson, 2000; Zhang and Mehvar, in press) that after intravenous administration, DMP is substantially sequestered in the liver and spleen, where it releases parent drug gradually. Additionally, after i.v. administration of equivalent doses, the immunosuppressive activities of DMP were both more intense and delayed relative to those after MP injection (Mehvar and Hoganson, 2000). Therefore, dextran conjugation of immunosuppressants appears to be a promising approach for delivery of these agents because it preferentially delivers the immunosuppressants to their site of action (Zhang and Mehvar, in press), increases the duration of action of a single dose by releasing the active drug slowly (Mehvar and Hoganson, 2000), and has a potential to reduce toxicity by limiting the distribution of the drug to other tissues (Zhang and Mehvar, in press).

It is generally believed that dextrans enter cells through fluid-phase endocytosis (Lake et al., 1985; Stock et al., 1989), a process which is expected to be concentration-independent or linear (Moss and Ward, 1991). However, our laboratory has shown (Mehvar et al., 1995) that the accumulation of high M_w dextrans in the liver and spleen may be modestly dose-dependent (nonlinear). Others (Nishikawa et al., 1992) have also suggested that the liver uptake of dextrans may be receptor mediated, and therefore, subject to saturation. Additionally, previous studies (Mehvar and Reynolds, 1993; Mehvar et al., 1994, 1995; Mehvar, 1997) have shown that the efflux of large M_w dextrans from the liver and spleen is limited. Therefore, the sequestration of dextrans in these tissues serves as a means of clearance of the macromolecule. Consequently, a nonlinear uptake of dextrans with large M_w into the liver and spleen resulted in a nonlinearity in the systemic clearance of the macromolecule (Mehvar et al., 1995). Because the pharmacokinetics of dextran-drug conjugates are mainly governed by the kinetics of the carrier dextran (Larsen, 1989), we hypothesized that the pharmacokinetics of DMP may be dose-dependent. Therefore, the dose-dependency of the pharmacokinetics of DMP in rats were studied, the results of which are reported here.

2. Materials and methods

2.1. Chemicals

Dextran with an average M_w of 73 000, 6α-methylprednisolone (MP), heparin sodium, dextranase (EC 3.2.1.11, from *Penicillium* sp., crude), and esterase (EC 3.1.1.1, from porcine liver, crude) were obtained from Sigma Chemical (St. Louis, MO). 6α-methylprednisolone 21-hemisuccinate (MPS) was purchased from Steraloids (Wilton, NH). Ketamine HCl (100 mg/ml, Fort Dodge Animal Health, Fort Dodge, IA), xylazine (20 mg/ml, Bayer Corporation, Shawnee, KS), and acepromazine maleate (10 mg/ml, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) were obtained from commercial sources. The anesthetic solution injected to rats was prepared by mixing ketamine, xylazine and acepromazine maleate (3:3:1 v/v/v) as described before (Harkness and Wagner, 1995). Sterile water (injectable, USP) was purchased from Abbott Laboratories (North Chicago, IL). For chromatography, HPLC grade acetonitrile (Mallinckrodt Chromar HPLC) was obtained from VWR Scientific (Minneapolis, MN). All other reagents were analytical grade and obtained through commercial sources.

Dextran-methylprednisolone succinate (DMP) was synthesized, purified, and characterized by modification of a previously reported method (McLeod et al., 1993) as described before (Mehvar, 1999). Based on a size-exclusion chromatographic method (Mehvar, 1999), the MP and MPS impurities in the conjugate powder were less than 0.1% (w/w) and the degree of substitution of the powder was 8 mg of MP per 100 mg of the powder.

2.2. Animals

All procedures involving animals used in this study were approved by our Institutional Animal Care and Use Committee. Adult male Sprague–Dawley rats (262–310 g) were obtained from Charles River Lab (Wilmington, MA) and housed in a 12-h light–dark cycle and constant temperature-controlled animal facility for at least 2 days prior to the experiments. The animals had free access to drinking water and rat chow before and during the course of experiments.

2.3. Surgery, dosing, and sample collection

The day before dosing, the left jugular vein of the rat was cannulated after an i.p. injection of 0.6 ml/kg of the anesthetic mixture. The cannula was made of a PE50 tubing with a silastic tip of 2.7–2.8 cm in length. After implantation, the catheter was flushed with an isotonic phosphate buffer solution containing heparin (100 units). Rats were then placed in individual metabolism cages and allowed to recover overnight.

A total of 25 rats were divided into five dosing groups ($n = 5$/group) of 2.5, 5.0, 10, 20 and 30 mg/kg of DMP (MP equivalent) with body weights (mean $\pm$ SD) of 280 ± 15, 279 ± 7, 277 ± 6, 283 ± 9, and 274 ± 21 g, respectively. Injection solutions of DMP were prepared daily by dissolving 31.1, 62.5, 125, 250, or 375 mg of DMP in 2 ml of sterile injection water, resulting in the MP equivalent concentrations of 1.25, 2.5, 5, 10, and 15 mg/ml, respectively, based on an 8% degree of substitution. The dosing solutions (2 ml/kg) were administered via the jugular vein cannula over 3 min. The blood samples (~ 0.25 ml) were then collected at 0 (before dosing), 0.25, 1, 2, 3, 5, 8, 12, 24, 36, 48 and 96 h. For the lowest dose (2.5 mg/kg), sampling was stopped at 24 h. However, two extra samples were taken at 0.5 and 4 h for this group. Assuming a blood volume of 6% of total body weight, the total volume of blood taken from rats during sampling was $15.1 \pm 0.7\%$ of the total blood volume.

Blood samples were centrifuged immediately in a pre-chilled and heparin-coated microcentrifuge tube, and the resultant plasma was stored at -80 °C until analysis. Previous studies (Mehvar, 2000b) have shown that DMP is stable under these conditions for at least 11 weeks.

Except for the 2.5 mg/kg dosing group, which was euthanized at 24 h, the animals were euthanized at 96 h by means of carbon dioxide, and liver and spleen were collected. Immediately after excision, the collected tissues were rinsed in ice-cold saline solution to remove excess blood. Afterwards, the tissues were blotted dry and then homogenized in three volumes of 2% glacial acetic acid solution. The dextran-methylprednisolone conjugate is reportedly (Mehvar et al., 2000b) stable in the presence of acetic acid. The whole homogenate was frozen at -80 °C until analysis.

2.4. Size-exclusion chromatographic analysis of dextran-methylprednisolone in plasma and spleen

The concentrations of DMP in plasma were measured directly using a size-exclusion chromatographic method reported recently (Mehvar, 2000b). Briefly, after the addition of 50 μl of 0.4 M phosphate buffer (pH 7.0) and 50 μl of methanol to 100 μl of the sample, the proteins were precipitated by the addition of 20 μl of a 20% (v/v) perchloric acid solution. The conjugate in the supernatant was then detected at 250 nm using a mobile phase of water:acetonitrile:acetic acid (75:25:0.2) run at a flow rate of 1 ml/min. The lower limit of quantitation of this assay was 2 μg/ml (MP equivalent) (Mehvar, 2000b).

For determination of the concentrations of DMP in the spleen homogenates, the plasma assay reported above was used with a modified mobile phase of 0.1 M KH_2PO_4:acetonitrile (75:25). The recovery of DMP from the spleen

homogenates was similar to that reported (Mehvar, 2000b) for the plasma samples (> 80%).

2.5. Analysis of dextran-methylprednisolone in liver by enzymatic hydrolysis

The concentrations of DMP in the liver homogenates could not be accurately measured using the direct size-exclusion chromatographic method explained above. This was because of the presence of a large endogenous peak eluting immediately before the DMP peak in the liver homogenate samples. Therefore, the DMP concentrations in the liver samples were determined indirectly by measurement of released MP after sequential enzymatic hydrolysis of DMP by dextranase and esterase as described here. To 50 μl of liver homogenate (pH 4.8) was added 100 μl of 0.0672 M phosphate buffer (pH 7.4), resulting in a pH of 6.75. After the addition of 20 μl of dextranase (0.75 unit/μl in water), the solution was gently mixed and incubated at 37 °C for 30 min. Afterwards, the pH of the mixture was brought up to 7.4 by the addition of 505 μl of a solution containing 0.0672 M phosphate buffer (pH 7.4) and 0.17 M NaOH (7:1.1 v/v). Twenty microliters of an esterase solution (5 units/μl in water) was then added to the mixture, which was incubated at 37 °C for an additional 24 h. The released MP concentration was then determined by a published (Mehvar et al., 2000b) reversed-phase HPLC method. The efficiency of the method in releasing MP was tested ($n = 3$) by subjecting homogenate samples containing 100 μg/ml DMP to the sequential hydrolysis and analyzing the released MP by HPLC (Mehvar et al., 2000b).

For the analysis of unknown samples, standards containing 2.5, 5, 10, 20, 40, 60, 80 and 100 μg/ml of DMP were prepared in blank homogenates and subjected to the same sequential hydrolysis and subsequent HPLC analysis as the unknown samples.

2.6. Analysis of data

Noncompartmental analysis (Gibaldi and Perrier, 1982) was used for the estimation of kinetic parameters. Because the first blood sample was taken 0.25 h after the drug administration, the concentration at time zero (immediately after the drug administration) was estimated from the exponential regression analysis of the first four concentration–time data which showed log linear decline ($r^2 = 0.91$–1.0). The area under the plasma concentration–time curve (AUC) was estimated using linear trapezoidal rule for the duration of sampling and extrapolated to infinity. The extrapolated AUCs were $\leqslant 10\%$ of the total AUC for all the doses. The apparent terminal rate constant (λ) was estimated from the log-linear portion of the plasma concentration–time curve (last three samples). Because of the limit of the detection of the assay (2 μg/ml), the plasma concentrations of DMP could not be measured up to the last sampling time (96 h) for all the doses. Therefore, the λ values for different doses could not be calculated from the same sampling times. Linear trapezoidal rule with end correction was also applied for estimation of the area under the first moment curve (AUMC). Clearance (Cl) was estimated by dividing the dose by AUC. Volume of distribution at steady state (V_{ss}) was estimated from the following equation based on statistical moment analysis (Gibaldi and Perrier, 1982):

$$V_{ss} = \frac{\text{Dose}\,\text{AUMC}}{\text{AUC}^2}. \quad (1)$$

The mean residence time (MRT) of DMP in the body for each dose was estimated by a reported (Jusko, 1989) method for systems with nonlinear elimination. In this method (Jusko, 1989), V_{ss} is first estimated for a low dose in the linear range. The true MRT value for each dose is then estimated by dividing this V_{ss} by Cl obtained for individual rats and doses.

The Michaelis–Menten parameters of the nonlinear elimination of DMP were estimated from the slope and intercept of a plot of MRT against the DMP dose based on the following equation (Jusko, 1989):

$$\text{MRT} = \frac{1}{2V_{MAX}}\text{Dose} + \frac{V_{ss}K_M}{V_{MAX}}, \quad (2)$$

where V_{MAX} and K_M are the maximum rate of elimination and the plasma concentration produc-

ing half of V_{MAX}, respectively. The estimated Michaelis–Menten parameters were then used to estimate the theoretical values of AUCs at different doses using the following equation (Jusko, 1989):

$$AUC = \frac{Dose}{V_{MAX}}\left(\frac{Dose}{2V_{ss}} + K_M\right). \quad (3)$$

The above calculations are based on the assumption that V_{ss} is dose-independent (Jusko, 1989), which is in agreement with previous data for the dextran macromolecules (Mehvar et al., 1995). The V_{ss} value used for the above calculations was from the lowest dose used in our studies (2.5 mg/kg), which is in the linear range (Section 3).

The percent dose found in the liver and spleen at 96 h was calculated from the tissue concentration of drug (C_t) and tissue weight (W_t) by ($C_t W_t 100$)/Dose. The concentrations of DMP in the liver and spleen were corrected for the residual blood (Mehvar et al., 1994) using the volume fractions (Bernareggi and Rowland, 1991) of 0.0572 and 0.321, respectively.

The statistical differences among different doses with regard to pharmacokinetic parameters were tested using ANOVA with subsequent Scheffe's *F*-test. The regression between MRT and dose was tested using regression analysis. All the tests were performed at a significance level of 0.05. Data are presented as mean ± SD.

3. Results

The dose-corrected plasma concentration–time courses of DMP after single i.v. doses of 2.5–30 mg/kg are presented in Fig. 1. The decline in the plasma concentrations of DMP was multiexponential for all the studied doses (Fig. 1). Additionally, the profiles for the 2.5 and 5.0 mg/kg doses were superimposable, suggesting linear pharmacokinetics of DMP within this dosage range. However, the dose-corrected plasma concentrations progressively increased for doses of 10, 20, and 30 mg/kg, suggesting nonlinear pharmacokinetics within the 10–30 mg/kg dosage range.

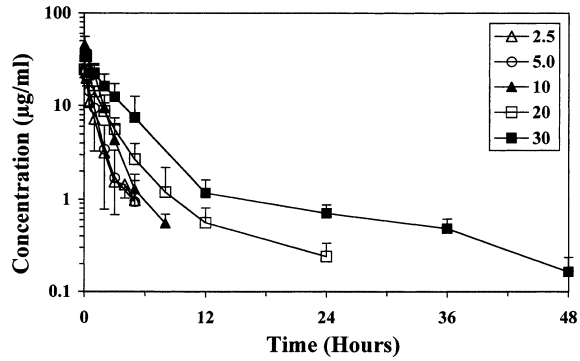

Fig. 1. The dose-corrected plasma concentration–time courses of DMP after single intravenous doses of 2.5 (△), 5.0 (○), 10 (▲), 20 (□), and 30 (■) mg/kg ($n = 5$ for each dose). Symbols represent the average values corrected for a dose of 1 mg/kg. SD values are presented as error bars.

The major pharmacokinetic parameters of DMP are presented in Table 1. Both clearance and the apparent terminal rate constant values were dose-dependent (Table 1). Generally, the clearance of DMP decreased with an increase in the dose ($P = 0.0002$); an increase in dose from 2.5 to 30 mg/kg resulted in more than five-fold decrease in the clearance value. In agreement with the clearance values, the terminal rate constant of

Table 1
Mean ± SD of major pharmacokinetic parameters of DMP after single intravenous doses (MP equivalent) of 2.5, 5.0, 10, 20, and 30 mg/kg to rats ($n = 5$ for each dose)

Dose (mg/kg)	Kinetic parameter		
	Clearance[a,*] (ml/h per kg)	V_{ss} (ml/kg)	$\lambda^{b,*}$ (h^{-1})
2.5	42.1 ± 11.0	78.8 ± 56.5	0.555 ± 0.326
5.0	45.3 ± 24.6	–[c]	0.765 ± 0.442
10	14.8 ± 2.9	–[c]	0.133 ± 0.064
20	17.3 ± 5.7	–[c]	0.117 ± 0.044
30	7.72 ± 1.84	–[c]	0.064 ± 0.020

[a] Significant differences between the dose of 2.5 vs. 10 and 30, and between 5 vs. 10, 20, and 30 mg/kg.
[b] Significant differences between the dose of 5 vs. 10, 20, and 30 mg/kg.
[c] Not calculated because of nonlinear clearance.
* Significant differences among the doses (ANOVA, $P < 0.001$).

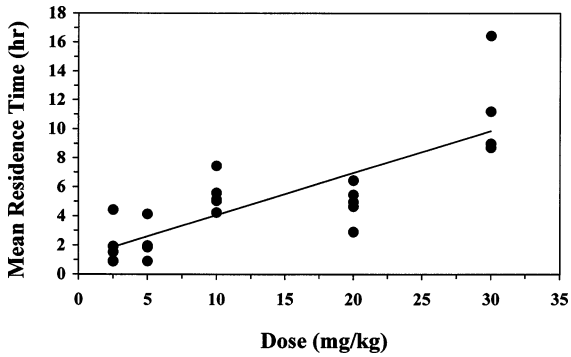

Fig. 2. The relationship between MRT of DMP and the administered dose for individual rats. The equation describing the relationship is $MRT = 0.291 \times Dose + 1.14$ ($P < 0.00001$; $r^2 = 0.67$).

DMP also significantly ($P = 0.0005$) decreased with an increase in the administered dose (Table 1).

The MRT values of DMP for different doses are depicted in Fig. 2. The mean $\pm$ SD values were 1.95 ± 1.45, 2.18 ± 1.19, 5.40 ± 1.20, 4.90 ± 1.30, and 10.8 ± 3.32 h for the 2.5, 5.0, 10, 20, and 30 mg/kg doses, respectively ($P < 0.0001$, ANOVA). There was a significant ($P < 0.00001$; $r^2 = 0.67$) relationship between the two parameters, with a slope of 0.291 and intercept of 1.14. The V_{MAX} and K_M values estimated (Jusko, 1989) from the slope and intercept values were 1.72 mg/h per kg and 24.9 μg/ml, respectively.

The experimental and predicted AUC values of DMP after different doses are presented in Fig. 3. The predicted AUCs were estimated from Eq. (3) using the Michaelis–Menten parameters obtained from Fig. 2. As expected from the clearance values (Table 1), the relationship between the AUC and dose was nonlinear, with higher doses resulting in more than proportional increases in the AUC values (Fig. 3).

The time course of release of MP from dextranase-mediated depolymerized DMP in the presence of esterase is demonstrated in Fig. 4. Dextranase enzyme resulted in a rapid degradation of dextran polymer as the peak representing DMP in the size exclusion chromatography completely disappeared within 5 min of the enzyme addition (data not shown). The release of MP

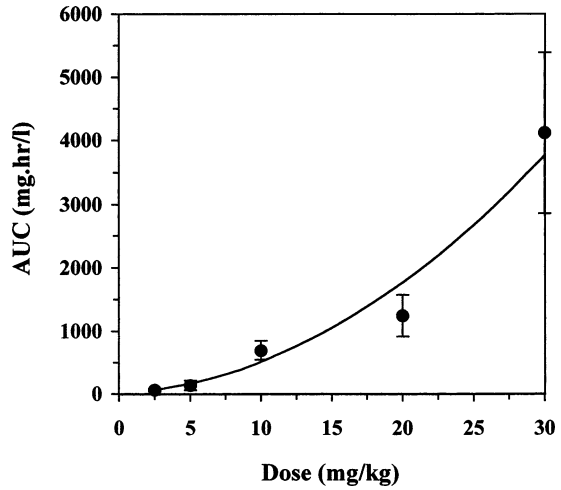

Fig. 3. The relationship between the plasma AUC of DMP and the administered dose ($n = 5$ for each dose). The symbols and bars represent the average and SD of experimental values, respectively. The line represents the theoretical relationship based on Eq. (3).

from the depolymerized DMP was, however, relatively slow with a half-life of ~ 5 h, reaching a plateau at 24 h (Fig. 4). The pretreatment of homogenates with dextranase was essential because no significant esterase-mediated release of MP was observed in the absence of dextranase. Nevertheless, the sequential hydrolysis of DMP using dextranase followed by esterase resulted in the release of $\approx 90\%$ of the conjugated MP within 24 h (Fig. 4). Therefore, liver homogenate samples

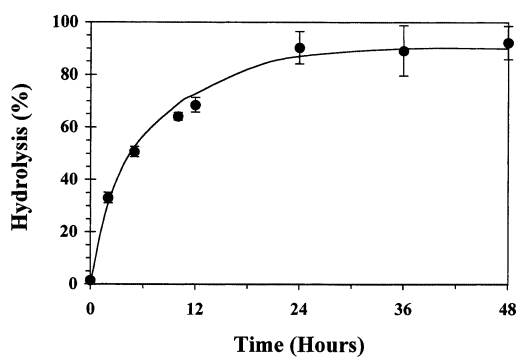

Fig. 4. The time course of release of MP from depolymerized DMP in the presence of esterase. The symbols are the average of three experiments, and bars represent SD values. The line is empirically drawn.

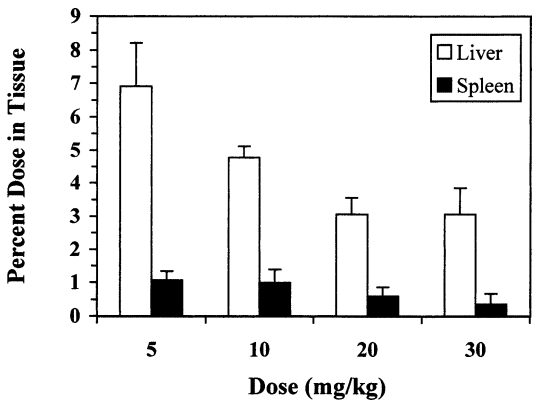

Fig. 5. The average amounts of DMP found in the liver (open bars) and spleen (closed bars) at 96 h after the administration of various doses of DMP. SD values are presented as error bars.

were subjected to this hydrolysis, and the concentrations of free MP were measured.

The percentages of DMP dose found in the liver and spleen at the end of sampling (96 h) are reported in Fig. 5 for doses of 5.0–30 mg/kg. Although the plasma levels of DMP were below the limit of quantitation at 96 h for all the doses (Fig. 1), substantial amounts of DMP were found in both the liver and spleen at this time (Fig. 5). The percentage of dose found in both the liver and spleen was dose-dependent ($P < 0.001$ and < 0.01 for the liver and spleen, respectively) with a trend towards lower percentages at higher doses (Fig. 5). However, it should be noted that the absolute amount of DMP in tissues increased with an increase in the dose. No parent drug was detected in either the liver or spleen at 96 h.

4. Discussion

Previous studies (Mehvar and Hoganson, 2000; Rensberger et al., 2000; Zhang and Mehvar, in press) have shown promising pharmacokinetic and pharmacodynamic characteristics for DMP as a prodrug of the immunosuppressive drug MP for targeted delivery to tissues such as spleen and liver. After i.v. administration, substantial amounts of the prodrug preferentially accumulated in the liver ($> 29\%$ of the dose) and spleen ($> 5.5\%$ of the dose) where the active MP was gradually released (Zhang and Mehvar, in press). In terms of concentrations, dextran conjugation resulted in 800- and 6000-fold increases in the AUC of the steroid in the liver and spleen, respectively, when compared with the values after the injection of the parent drug (Zhang and Mehvar, in press). The preferential delivery of the steroid to the spleen by the prodrug was associated with a significant increase in the intensity and duration of the immunosuppressive effects of the steroid (Mehvar and Hoganson, 2000). Therefore, additional investigations into the action and disposition of the prodrug are warranted.

The plasma pharmacokinetic data presented in this article demonstrate that the clearance of the prodrug decreases with an increase in dose (Table 1). The nonlinearity in clearance is also reflected in a decrease in the apparent terminal rate constant (Table 1) and an increase in the MRT (Fig. 2) when the dose is increased from 2.5 to 30 mg/kg.

The clearance of DMP is expected to occur mainly via nonrenal pathways. This is because the large size of the carrier (70 000) excludes its excretion via glomerular filtration (Mehvar and Shepard, 1992; Mehvar et al., 1994); only 5% of a 5-mg/kg dose of a fluorescein-labeled dextran with a M_w of 70 000 was excreted into urine of rats (Mehvar et al., 1994). For these high M_w dextrans, hepatic accumulation appears to be a major pathway of elimination because the accumulated dextran is trapped in the liver cells where it is gradually eliminated (Mehvar et al., 1994, 1995). Similarly, tissue accumulation and further degradation of DMP and release of MP are reported as major pathways for the clearance of DMP (Zhang and Mehvar, in press). Therefore, the observed nonlinear clearance of DMP (Table 1) is, most likely, due to a nonlinearity in the tissue, especially the liver, clearance of the prodrug.

Contrary to the general assumption of fluid-phase endocytosis for the hepatic uptake of dextrans (Lake et al., 1985; Stock et al., 1989), we have shown (Mehvar et al., 1995) that at low doses, the hepatic clearance of high M_w dextrans are > 40-fold higher than the rate of fluid-phase endocytosis in the rat liver (0.333 ml/h per kg)

(Munniksma et al., 1980). This suggests a significant contribution of other mechanisms, in addition to the concentration-independent fluid-phase endocytosis, to the hepatic uptake of these macromolecules. Indeed, others (Nishikawa et al., 1992) have suggested a receptor-mediated mechanism for the hepatic uptake of dextrans in mice. Therefore, a dose-dependent uptake of DMP into the liver and possibly other tissues may be responsible for the observed nonlinearity in the plasma profile of DMP. This postulate is consistent with the observed reduction in the percentage of dose of DMP found in the liver and spleen at higher doses of the prodrug (Fig. 5).

In the liver, it was necessary to regenerate MP from the conjugate before measuring its concentrations. This was due to the fact that the freeze-thaw of the liver homogenate resulted in appearance of a relatively large peak interfering with the DMP peak in the size-exclusion chromatographic method developed for the plasma samples (Mehvar, 2000b). Although complete alkaline hydrolysis of DMP is very fast (< 5 min) in aqueous solutions (Mehvar, 1999), this method resulted in relatively slow hydrolysis in the liver homogenate samples. Because MP is unstable under basic conditions (Oshima et al., 1980), a slow alkaline hydrolysis of the liver samples resulted in an initial increase and subsequent decrease in the concentrations of MP with time. Therefore, it became necessary to use an enzymatic method for the release of MP from DMP.

The sequential metabolism of DMP by dextranase and esterase reported here is based on the proposed mechanisms of release of non-steroidal (Larsen et al., 1989) and steroidal (McLeod et al., 1994) drugs from dextran carriers in the lower parts of the gastrointestinal tract. In a series of studies on the ester conjugates of non-steroidal antiinflammatory drugs with dextrans, Larsen and colleagues (Harboe et al., 1989; Larsen et al., 1989; Larsen and Jensen, 1991) showed that despite presence of esterases in the upper parts of the gastrointestinal tract, the conjugates released the drugs mostly in the colon. This was attributed (Larsen et al., 1989) to the large molecule of dextrans, preventing the action of esterases on the ester bonds in the upper part of the gastrointestinal tract. However, in the colon, bacterial dextranases would depolymerize dextrans, resulting in lower M_w fragments that can be hydrolyzed by esterases to release the drug (Larsen et al., 1989). Similar findings were reported (McLeod et al., 1993, 1994) for steroid–dextran conjugates investigated for colonic delivery of steroids. In agreement with these studies, when esterases alone were used in our studies, no appreciable release of MP was noticed. However, initial depolymerization with dextranase resulted in an almost complete release of MP as a result of subsequent esterase treatment (Fig. 4).

The enzymatic hydrolysis of DMP, reported here for indirect measurement of MP, would also provide some insights into the possible mechanisms for the reported (Zhang and Mehvar, in press) release of MP in tissues such as the liver and spleen after the DMP injection. Based on the data presented here, a significant enzymatic release of MP cannot take place on the intact DMP; in the absence of depolymerization, MP can be released only by a slow chemical hydrolysis (McLeod et al., 1993; Mehvar et al., 2000a). However, in addition to the colon, dextranases are reportedly (Rosenfeld and Lukomskaya, 1957) present in organs such as the liver and spleen. Indeed, in vivo studies have shown that the M_w of the carrier dextrans decreases with time in the liver of both rats (Mehvar et al., 1995) and mice (Kaneo et al., 1997). Therefore, it is expected that a depolymerization of DMP in these tissues would facilitate further enzymatic action of esterases in releasing the active drug. Nevertheless, the potential toxicity of the prodrug accumulated in these tissues after multiple dose regimens should be investigated in future studies.

In conclusion, the effects of dose on the pharmacokinetics of a dextran-methylprednisolone conjugate were studied in rats. It was shown that the clearance of the conjugate decreases with an increase in dose. The nonlinearity in the clearance of the conjugate appears to be related, at least in part, to a nonlinearity in the uptake and accumulation of the prodrug in tissues such as the liver and spleen.

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