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Dextran-Methylprednisolone Succinate as a Prodrug of Methylprednisolone: Plasma and Tissue Disposition

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Dextran-Methylprednisolone Succinate as a Prodrug of Methylprednisolone: Plasma and Tissue Disposition[1]

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Abstract

Plasma and tissue disposition of a macromolecular prodrug of methylprednisolone (MP), dextran (70 kDa)-methylprednisolone succinate (DMP), was studied in rats. Single 5-mg/kg doses of DMP or unconjugated MP were administered into the tail veins of different groups of rats (n = 4/group/time point). Blood (cardiac puncture) and tissues (liver, spleen, kidney, heart, lung, thymus, and brain) were collected at various times after DMP (0-96 h) or MP (0-2 h) injections. Concentrations of DMP and MP in samples were analyzed by size-exclusion and reversed-phase HPLC methods, respectively. Conjugation of MP with 70-kDa dextran resulted in 22-, 300- and 30- fold decreases in the steady state volume of distribution, clearance, and terminal plasma rate constant of the steroid, respectively. As for tissue distribution, the conjugate delivered the steroid primarily to the spleen and liver as indicated by 19- and 3-fold increases, respectively, in the tissue:plasma AUC ratios of the steroid. On the other hand, the tissue:plasma AUC ratios of the prodrug in other organs were negligible. Active MP was released from DMP slowly in the spleen and liver, and AUCs of the regenerated MP in these tissues were 55- and 4.8-fold, respectively, higher than those after the administration of the parent drug. In contrast, no parent drug was detected in the plasma of DMP-injected rats. These results indicate that DMP may be useful for the targeted delivery of MP to the spleen and liver where the active drug is slowly released.

INTRODUCTON

Glucocorticoids, such as methylprednisolone (MP), have been widely used for prevention of graft rejection in organ transplantation.[1-4] However, even at moderate doses, chronic administration of these steroids results in many side effects such as diabetes, hypertension, cushing syndrome, and osteoporosis.[5] Therefore, selective delivery of glucocorticoids to the immune system such as the liver and spleen would be advantageous for improving the efficacy and reducing side effects of these drugs in organ transplantation. Indeed, studies[6,7] using liposomal formulations of MP have shown that enhanced delivery of the steroid to the spleen would result in an improved survival rate in experimental organ transplantation.

As an alternative to liposomes, polymers such as dextrans may be used as macromolecular carriers for targeted delivery of immunosuppressive drugs to their site of action (i.e. reticuloendothelial system). Dextrans are glucose polymers which have long been used as plasma volume expanders.[8] Due to their rich and easily modifiable hydroxyl groups and their low immunogenicity, dextrans have been extensively investigated as macromolecular carriers to deliver drugs to target organs[9,10] or tumor cells.[11-13] Recently, our laboratory showed[14,15] that the tissue accumulation of dextrans was influenced by the molecular weight (M_w) of the macromolecule. For example, dextran with M_w of 70 kDa (dextran-70) was mainly accumulated in the liver and spleen.[15] In addition, it has been suggested[16] that the kinetics of dextran conjugates are mainly governed by dextran carriers. Therefore, we hypothesized that dextran-70 may be an appropriate candidate for the selective delivery of MP and other immunosuppressive drugs to the liver and spleen.

Dextran-methylprednisolone succinate (DMP), a conjugate of MP and dextran containing two ester bonds, was previously synthesized[17] using succinic acid as a linker between the

polymer and MP (Scheme I).[10] Hydrolysis studies[10,18] showed that at physiological pH, DMP is slowly hydrolyzed at both ester bonds (Scheme I), resulting in the formation of MP and methylprednisolone succinate (MPS), the latter being subsequently converted to MP. Therefore, the present investigation was designed to determine the plasma and tissue disposition of DMP and its hydrolysis products after the intravenous administration of the conjugate to rats. For comparison, the plasma and tissue disposition of MP after the injection of an equivalent dose of the parent drug was also investigated. The hypothesis of this investigation was that the conjugation of MP with dextran 70 kDa would result in targeted delivery of MP to the reticuloendothelial system.

EXPERIMENTAL SECTION

Materials

Dextran-70, 6α-methylprednisolone (MP), and internal standard (triamcinolone acetonide) were obtained from Sigma Chemical (St. Louis, MO). 6α-methylprednisolone 21-hemisuccinate (MPS) and methylprednisone (MPN) were purchased from Steraloids (Wilton, NH). For chromatography, HPLC grade acetonitrile (Mallinckrodt Chromar HPLC) was obtained from VWR Scientific (Minneapolis, MN). All other reagents were analytical grade. The DMP conjugate was synthesized from dextran-70 and MPS based on published methods.[10,17] The degree of substitution of MP on DMP (8%, w/w) was determined as described before.[17] The conjugation drastically changed the water solubility of MP from negligible (parent drug) to more than 20 mg/mL (250 mg/mL of the conjugate).

Animals

The procedures involving animals used in this study were consistent with the guidelines set by the National Institute of Health (NIH publication #85-23, revised 1985) and approved by our Institutional Animal Care and Use Committee. Adult male Sprague Dawley rats (201-250g) were obtained from Charles River Lab (Wilmington, MA) and housed in a 12-h light-dark cycle and temperature-controlled facility 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. A total of 56 animals were used for this study. Fifty-two rats were divided into two groups. One group (28 rats) was treated with DMP, and another group (24 rats) was treated with MP. The remaining four rats were used as organ donors for blank samples. The mean ± SD of the body weights of rats were 223 ± 11 and 218 ± 13 g for DMP- and MP-injected groups, respectively.

Dosing and Sample Collection

Dosing solution of DMP was prepared by dissolving 62.5 mg DMP in 1 mL HPLC water. Based on a degree of substitution of 8%,[17] the strength of this solution was equivalent to 5 mg/mL of MP. Dosing solution of MP (5 mg/mL) was prepared as reported before.[19]

Under mild ether anesthesia, single 5-mg/kg (MP equivalent) doses of MP or DMP were administered into the tail veins of rats. At various times after dosing, animals were sacrificed by means of carbon dioxide, and liver, spleen, right kidney, heart, lung, thymus, and brain were collected. Blood samples were also withdrawn by cardiac puncture. The samples were collected at the following times: 1 min and 2, 5, 12, 24, 48, and 96 hours for DMP-injected rats, and 1, 10, 20, 40, 60, and 120 min for MP-injected rats (n = 4/group/time point).

Immediately after excision, the collected tissues were rinsed in ice-cold saline solution to remove excess blood. Afterwards, the tissues were blotted dry and kept frozen until analysis. After centrifugation of the blood in a pre-chilled and heparin-coated microcentrifuge tube, the resultant plasma sample was divided into two portions. One hundred μL of plasma sample was transferred to a silicon-coated microcentrifuge tube for DMP analysis.[20] For unconjugated MP analysis, 500 μL of plasma sample was transferred to a pre-chilled glass tube containing 100 μL of a 10% acetic acid solution to prevent DMP hydrolysis in vitro.[21] Both plasma and tissue samples were kept frozen at -80^{o}C until analysis.

Sample Analysis

The concentrations of DMP in plasma were measured by using a size-exclusion HPLC method.[20] Using a 100 µL sample, the assay has a lower limit of quantitation of ≤ 2 µg/mL with intra- and inter-run CVs of < 6% and error values of < 5%. The concentrations of MPS, MP, and MPN in plasma were analyzed simultaneously by using a reversed-phase HPLC method.[21] Utilizing a 0.5 mL sample, the lower limit of quantitation of the reversed-phase assay is ≤ 0.1 µg/mL for all the analytes with intra- and inter-run CVs of <16% and error values of < 8% for all the components.

Organs were first homogenized in 3 volumes of 2% glacial acetic acid solution, and the resultant homogenates were used for drug measurements. Previous studies[18] have shown that in the presence of acetic acid, no hydrolysis of DMP occurs during the storage (up to three weeks at -80^{o}C) and sample analysis. For measurement of MPS, MP, and MPN in the tissues, the homogenates were treated similar to plasma[21] with one exception: instead of a mobile phase of 0.1 M acetate buffer (pH 5.7): acetonitrile (77:23), which was used for plasma,[21] the mobile phase was 0.1 M phosphate buffer (pH 5.8) :acetonitrile (77:23) for the tissues. The slight change in the aqueous part of the mobile phase was necessary to separate MPS from a small endogenous

peak in some tissue homogenates. The recovery of the analytes from all the tissues was very similar to that reported[21] for the plasma (>80%).

For measurement of DMP in tissue homogenates, a modified version of the previously reported[20] plasma assay was used. Briefly, to 100 µL of tissue homogenates were added 50 µL of 0.4 M phosphate buffer (pH 7.0), 50 µL of methanol, and 20 µL of perchloric acid (70%). After a brief vortex-mixing and centrifugation, 170 µL of the supernatant was transferred to a new microcentrifuge tube, and DMP was precipitated by the addition of 1 mL ethanol. The supernatant after centrifugation was decanted and the tube dried under a nitrogen stream. The residue was then dissolved in 200 µL 0.1 M KH_2PO_4: acetonitrile (65:35), and 100 µL was injected into HPLC. The conjugate was detected (λ = 250 nm) after analysis of samples on a size-exclusion column (Polysep-GFC; Phenomenex, Torrance, CA, USA) with a mobile phase of 0.1 M KH_2PO_4: acetonitrile (75:25, v/v), run at a flow rate of 1 mL/min. The lower limit of quantitation of this assay was 1 µg/mL based on a 100-µL homogenate sample, and the tissue recovery from the homogenates was ≥70%.

Pharmacokinetic Analysis

Non-compartmental analysis was performed by using WinNonlin™3.1 computer program (Pharsight Co.; Mount View, California). Terminal elimination rate constant (λ_z) was estimated from the log-linear portion of the plasma or tissue concentration-time courses. Area under the plasma or tissue concentration-time curve (AUC) was estimated from the average plasma concentrations at different time points using linear trapezoidal rule with extrapolation to infinity. Other estimated pharmacokinetic parameters included: mean residence time (MRT), apparent total body clearance (CL), volume of distribution at steady-state (V_{ss}), terminal volume of distribution (V_z), maximum observed drug concentration (C_{max}), and time to reach C_{max} (T_{max}).

The maximum concentrations of DMP or MP in plasma (C_0) after the injection of the conjugate or parent drug were assumed to be the same as the concentrations at the first sampling time (1 min). The percentage of the total dose in plasma at different times was calculated from the plasma concentration (C_p) and plasma volume (V_p) by $\frac{C_p \cdot V_p \cdot 100}{Dose}$. The percentage of the total dose in each analyzed tissue was calculated from the tissue concentration of drug (C_t) and experimental tissue weight (W_t) by $\frac{C_t \cdot W_t \cdot 100}{Dose}$. For kidneys, percentage of the total dose found in the right kidney was multiplied by two to account for both kidneys. The concentrations of drugs in tissues were corrected[15] for the residual blood using the volume fraction (V_B) of blood in different organs; V_B values of 0.0135, 0.061, 0.0459, 0.0572, 0.175, 0.321, and 0.0088 were used for brain, heart, kidney, liver, lung, spleen, and thymus, respectively.[22]

Statistical Analysis

Because of destructive sampling procedure used for the collection of blood and tissues from different animals at each time point, the composite kinetic parameter AUC could not be obtained for individual rats.[15] Therefore the variance of AUC was estimated by a reported[23,24] procedure based on the standard error of mean and number of samples at each time point. The pairwise comparison of AUCs was then carried out at an α level of 0.05 and a Bonferroni-adjusted α of 0.05 or 0.0167 for pairwise comparison of two (1 comparison) or three (3 comparison) means, respectively. The critical values of Z (Z_{crit}) for the two-sided test using the Bonferroni-adjusted α of 0.05 and 0.0167 were 1.96 and 2.39, respectively, and the observed Z (Z_{obs}) was calculated as reported before.[23,24] A Z_{obs} value > Z_{crit} was used as an indication of a significant difference between the AUCs.

The differences among animal groups in their kinetic parameters which could be estimated for individual rats (e.g., C_{max} and C_0) were determined using a two-tailed unpaired t test (for comparison of 2 means) or ANOVA with subsequent Scheffe' F test (for comparison of 3 means) at a significance level (α) of 0.05. When possible, data are presented as mean ± SD.

RESULTS

Plasma Pharmacokinetics

Figure 1 depicts the mean plasma concentration–time courses of DMP and MP after the injection of equivalent doses of the conjugated or unconjugated drug to rats. After the injection of the unconjugated MP, the drug was eliminated rapidly and could not be detected at ≥ 2 h (Fig. 1). However, relatively high concentrations of DMP were detected in plasma until 24 h after the injection of the conjugate. Whereas the decline in the MP concentrations in plasma was apparently mono-exponential during the 1-h sampling period, DMP concentrations declined multi-exponentially (Fig. 1). Interestingly, no unconjugated drug was detected in plasma of DMP-injected rats.

Table 1 summarizes the estimated plasma pharmacokinetic parameters after MP and DMP injections. Attachment of MP to dextran-70 resulted in significant changes in the plasma disposition of the drug. The steady-state volume of distribution, total body clearance, and terminal elimination rate constant of DMP were 22-, 300- and 30-fold lower than the corresponding values for the parent drug (Table 1).

After the injection of DMP, no MPS, MP, or the metabolite MPN was detected in the plasma of animals. Additionally, the concentrations of the metabolite MPN were below the level of quantitation of the assay (0.1 μg/mL)[21] after the administration of the unconjugated MP.

Tissue Pharmacokinetics

After the injection of DMP, the intact conjugate or regenerated MP was not found in kidney, heart, lung, thymus or brain. However, high concentrations of DMP and regenerated MP were found in the liver and spleen (Figure 2). The high concentrations of DMP in these tissues persisted up to the last sampling time (96 h). In addition, after the injection of DMP, the concentrations of the regenerated MP could be measured up to 24 h in the liver and spleen (Figure 2). The relatively high concentrations of the regenerated MP in the liver and spleen after DMP injection (Fig. 2) suggest that the conjugate releases MP in these tissues. However, the concentrations of MPS and MPN were below the limit of quantitation of the assay (0.1 μg/mL)[21] in these tissues. In contrast to the profiles of MP regenerated after DMP injection, the hepatic and splenic concentrations of MP after the injection of the unconjugated MP declined rapidly and could not be detected beyond 2 h (Fig. 2).

The liver and spleen pharmacokinetic parameters for DMP- and MP-injected rats are summarized in Table 2. Comparing DMP kinetics (DMP-injected rats) with those of MP (MP-injected rats), the following changes in the tissue pharmacokinetic parameters were observed: conjugation of MP to dextran-70 resulted in 800- and 6000-fold increases in the AUCs of the steroid in the liver and spleen, respectively. Additionally, values of MRT for DMP in the liver and spleen were, respectively, 48- and 230-fold higher than those for MP (Table 2). Conjugation also resulted in a substantial decrease in λ_z values in both spleen and liver (Table 2).

Comparing the MP regenerated from DMP (DMP-injected rats) with MP after the injection of the unconjugated drug (MP-injected rats), the following changes in the tissue pharmacokinetic parameters were observed: conjugation to dextran increased absolute availability (i.e., AUC) of the unconjugated MP to the tissue by 4.8-fold in the liver and 55-fold

in the spleen (Table 2). In addition, the terminal rate constants of the regenerated MP in both spleen and liver were substantially smaller than those after the injection of MP (Table 2).

The percentages of the total dose found in plasma and different tissues as DMP or MP are depicted in Figure 3 after MP or DMP injections. For DMP (DMP-injected rats), percentage of the total dose in plasma initially decreased rapidly from 74% to 4.9 % within the first 2 h after the injection. Thereafter, the decline in the percentage of the dose in plasma became slower. Percentage of the total dose in the liver as DMP first showed an increase from zero to a maximum of 29% at 2 h and then gradually decreased, reaching a value of 4.2% at the last sampling time (96 h). The time course of the percentage of the total dose in the spleen as DMP paralleled that of DMP in the liver with a maximum of 5.5% at 12 h (Fig. 3).

For MP regenerated in DMP-injected rats (Figure 3, top), the percentage of the total dose in the liver reached a maximum value of 0.55% at 5 h, then decreased to 0.089% at 24 h. In the spleen, the highest percentage of the total dose as regenerated MP was found at 12 h after DMP injection (0.17%), and then the percentage decreased to 0.050 % at 24 h (Fig. 3, top).

In contrast to the primary delivery of DMP to the spleen and liver, MP was found in all the studied organs, except brain, after the injection of the parent drug (Fig. 3, bottom). The first sampling time immediately after dosing with MP (1 min) contained the highest percentages of MP in tissues (Fig. 3, bottom). The percentage values were highest in the liver (3.2%), followed by plasma (1.3%), kidney (0.97%), lung (0.84%), heart (0.67%), spleen (0.48%), and thymus (0.088%). Thereafter, the percentages in all the studied tissues declined very rapidly and became undetectable at 2 h after the injection of the free steroid (Fig. 3, bottom).

DISCUSSION

In rats, MP is a drug with a relatively high volume of distribution.[19] In addition, it is reported[25] that MP distributes to organs such as liver, spleen, lung, heart, muscle, kidney, and thymus, achieving higher concentrations in most of these tissues than in plasma. Our results in MP-injected rats (Table 1 and Fig. 3) are in agreement with these reports.[19,25] Conjugation of MP with dextran-70 drastically altered the distribution of the steroid by converting MP from a large volume of distribution drug (2290 mL/kg) to a prodrug with a small volume of distribution (102 mL/kg) (Table 1). In addition to reducing the volume of distribution of MP, conjugation with dextran resulted in a primary distribution of the prodrug into the liver and spleen (Table 2 and Fig. 3) with negligible distribution to any other studied tissues.

As for clearance, MP has a high and dose-dependent clearance in rats.[19,26] Conjugation to dextran-70 converted MP from a high clearance drug (125 mL/min per kg) to a prodrug with a very low clearance (0.413 mL/min per kg). In a previous study,[19] clearance values of 70 and 37 mL/min per kg were reported after 10- and 50-mg/kg doses of MP. Our higher clearance value of 125 mL/min per kg (Table 1) obtained after a lower dose (5 mg/kg) of MP is consistent with the nonlinearity[26] in the clearance of the steroid. Nevertheless, a clearance of 125 mL/min per kg is >2 fold greater than the rat liver blood flow of ~ 55 mL/min per kg,[27] suggesting that MP is also eliminated by extrahepatic pathways in this species.

The pharmacokinetics and tissue distribution pattern of DMP (Tables 1 and 2) resemble those[14,15] of the carrier polymer dextran-70. The reported[15] clearance of 0.5 mL/min per kg for fluorescein-labeled dextran-70 (FD-70) is very close to that of DMP estimated in our present study (0.4 mL/min/kg). However, V_{ss} of DMP (102 mL/kg) appears to be larger than that estimated[14] for FD-70 (62 mL/kg). The larger V_{ss} of DMP, compared with FD-70, is not

unexpected because dextrans are very water soluble macromolecules[28]. Therefore, conjugation with MP, a lipophilic drug with an octanol:water partition coefficient of ~70,[19] is expected to increase the lipophilicity of the carrier and possibly facilitate its distribution to tissues. Nevertheless, the significant distribution of DMP to the spleen and liver (Fig. 2) and lack of substantial distribution to other tissues are consistent with the distribution behavior of the carrier dextran.[15]

The tissue:plasma AUC ratio of the active drug is a more appropriate measure of the targetability of a drug to specific tissues than the absolute tissue concentrations or AUCs. Recent studies[29] in our laboratory demonstrated that DMP by itself lacks a significant immunosuppressive activity and should release MP in order to be effective. Therefore, a comparison of the tissue:plasma AUC ratios of the unconjugated MP after the administration of MP and DMP should determine the usefulness of dextran conjugation for targeted delivery of the steroid. After DMP injection, no parent drug was detected in plasma, whereas relatively high concentrations of the parent drug were regenerated from the conjugate in the liver and spleen (Table 2 and Fig. 2). Consequently, the actual liver or spleen:plasma AUC ratios of the parent drug after DMP injection could not be calculated. However, lack of detection of parent drug in plasma, after DMP injection, indicates a high degree of targeted delivery to the spleen and liver, compared with liver or spleen:plasma AUC ratios of <3 estimated after the injection of the unconjugated MP (Table 2).

The lack of detection of the parent drug in plasma after the injection of DMP is consistent with an in vitro study[18] demonstrating that DMP is relatively stable in rat blood with a hydrolysis half life of ~ 25 h. Further, it was suggested[18] that the hydrolysis of DMP in blood occurs via chemical hydrolysis rather than enzymatic hydrolysis by esterases present in blood.

The lack of enzymatic hydrolysis of ester conjugates of dextrans has been attributed to the large size of dextrans of high M_w (e.g., dextran 70).[16] However, it has been suggested[16] that lower M_w dextran-drug ester conjugates are susceptible to enzymatic hydrolysis. Because dextranase enzymes, which are responsible for the depolymerization of dextrans, are reportedly[30] not present in blood, only chemical hydrolysis is expected in this media. On the other hand, the highest concentrations of dextranases have been found in tissues such as the liver and spleen.[30] Therefore, dextranases may reduce the M_w of DMP in these tissues, making them more susceptible to enzymatic hydrolysis by the esterases and subsequent release of the parent drug, as observed in our studies (Fig. 2).

In a series of studies,[6,25,31-33] Jusko and colleagues prepared a liposomal formulation of MP and investigated the pharmacokinetics and pharmacodynamics of the formulation. The liposomal formulation selectively delivered the drug to the reticuloendothelial system with a 77-fold increase in the spleen:plasma AUC ratio when compared with the parent drug.[31] Additionally, it was shown that the liposomal formulation enhanced the tissue receptor occupancy[31] and immunosuppressive effects[6,32] of the steroid, hence improving survival rates in an experimental heart transplantation model.[6,34] Despite these promising results, the use of liposomes may not be ideal for delivery of all immunosuppressive drugs. This is because of potential problems with the relatively short stability of most liposomal formulations and the relatively limited drug load which may require higher than acceptable lipid loads injected to the patients. The targeted delivery of MP using the dextran prodrug approach reported here is an alternative to the use of liposomes for the delivery of the steroids and other immunosuppressive drugs to the immune system.

Dextrans have also been investigated recently[35] as macromolecular carriers for the delivery of the immunosuppressive drug tacrolimus (FK-506). In this study, tacrolimus conjugated to a negatively charged (carboxymethyl) dextran with a M_w of 70 kDa achieved a plasma AUC which was 2000-fold higher than that of the free drug. However, the spleen accumulation of the conjugate was only modestly higher than that after the parent drug administration. The apparent difference between dextran-tacrolimus[35] and dextran-MP (present study) in their splenic accumulation is most likely due to the differences in the electric charge of the carriers; whereas Yura et al.[35] used a negatively charged dextran for tacrolimus conjugation, we used a neutral dextran for conjugation with MP. Previous studies[36] have shown that the negatively charged dextrans achieve higher plasma concentrations and lower tissue (e.g., liver and spleen) accumulations, compared with neutral or positively charged dextrans. Nevertheless, dextran macromolecules appear to be suitable for improving the pharmacokinetics of a variety of immunosuppressive drugs.

In an attempt to reduce the toxicity and increase the effectiveness of immunosuppressants, local immunosuppression at the site of transplantation has been advocated.[37] This strategy is based on recent evidence indicating that in addition to the inhibition of the systemic immune system (such as inhibition of splenic lymphocytes), the inhibition of intragraft immune events at the site of transplantation is a major determinant of graft survival.[1,37] Therefore, the relatively high concentrations of DMP in the liver, shown in the present study, may also be advantageous for local immunosuppression in the case of liver transplantation.

In conclusion, conjugation of methylprednisolone with dextran 70 kDa drastically altered the pharmacokinetics of the steroid in rats. The conjugate was relatively stable in plasma and

primarily accumulated in the reticuloendothelial system (e.g., the liver and spleen) where it gradually released the parent drug. Dextran conjugation may be an effective strategy for targeted delivery of MP and other immunosuppressive agents to their site of action.

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Table 1— Plasma Pharmacokinetic Parameters (Mean ± SD) of unconjugated (MP) and Dextran-Conjugated (DMP) Methylprednisolone after a Single iv Dose (5 mg/kg, MP Equivalent) of MP or DMP

Parameter	MP-Injected Rats	DMP-Injected Rats[a]
	MP	DMP
C_o, μg/mL	2.11 ± 0.47[†]	120 ± 31
V_z, mL/kg	2280[b]	226[b]
V_{ss}, mL/kg	2290[b]	102[b]
AUC, μg h/mL	0.665 ± 0.048[†]	202 ± 17
Cl, mL/min per kg	125[b]	0.413[b]
MRT, h	0.305[b]	4.10[b]
λ_z, h^{-1}	3.30[b]	0.110[b]
$T_{1/2(\lambda z)}$, h	0.210[b]	6.32[b]

[a] No MP was detected in plasma after DMP injection. [b] Standard deviations could not be determined because of destructive sampling method. [†] Significantly different ($p < 0.05$) from the corresponding value for the DMP-injected rats.

Table 2—Liver and Spleen Pharmacokinetic Parameters (Mean ± SD) of Unconjugated (MP) and Dextran-Conjugated (DMP) Methylprednisolone after a Single iv Dose (5 mg/kg, MP Equivalent) of MP or DMP

Parameter	Liver			Spleen		
	MP-Injected Rats	DMP-Injected Rats		MP-Injected Rats	DMP-Injected Rats	
	MP	DMP	Regenerated MP	MP	DMP	Regenerated MP
AUC, µg h/g	1.94 ± 0.23[†]	1540 ± 76[†]	9.34 ± 0.89[†]	1.65 ± 0.14[‡]	9700 ± 731[‡]	90.6 ± 10.2[‡]
Tissue:Plasma AUC ratio	2.92[a]	7.62[a]	-[b]	2.48[a]	48.0[a]	-[b]
MRT, h	1.00[a]	47.5[a]	12.1[a]	0.325[a]	74.5[a]	15.5[a]
λ_z, h^{-1}	0.954[a]	0.0193[a]	0.0996[a]	2.91[a]	0.0131[a]	0.126[a,c]
$C_{max,}$ µg/mL	3.67 ± 3.25[¶]	33.5 ± 9.2[¶]	0.637 ± 0.152[¶]	5.60 ± 1.35[§]	106 ± 28[§]	4.38 ± 1.71[§]
$T_{max,}$h	0	2	5	0	12	12

[†] Significantly different from the liver AUC values for the other two means. [‡] Significantly different from the spleen AUC values for the other two means. [¶] Significant differences for liver C_{max} values between the MP and DMP groups and between DMP and Regenerated MP groups. [§] Significant differences for spleen C_{max} values between the MP and DMP groups and between DMP and Regenerated MP groups. [a] Standard deviations could not be determined because of destructive sampling method. [b] Could not be determined because no parent drug was found in plasma after DMP injection. [c] Based on two samples only.

Legends for Figures

Scheme 1. Chemical structure of dextran-methylprednisolone succinate

Figure 1. Plasma concentration–time courses of conjugated (DMP) and unconjugated (MP) methylprednisolone after iv administration of single 5-mg/kg doses (MP equivalent) of MP or DMP. Standard deviation values are shown as error bars (n = 4 for each point).

Figure 2. Liver (top) and spleen (bottom) concentration–time courses of parent (MP) and/or conjugated (DMP) methylprednisolone after iv administration of single 5-mg/kg doses (MP equivalent) of MP or DMP. Standard deviation values are shown as error bars (n = 4 for each point). The insets depict the tissue concentration-time courses of the unconjugated MP after the injection of the parent drug (open circles) or the dextran-conjugated steroid (closed circles).

Figure 3. The percentage of the total dose of the conjugated (DMP) and/or unconjugated (MP) methylprednisolone found in plasma and sampled tissues at various times after the iv administration of DMP (top) or parent drug (bottom). For comparison purposes, the time axis is the same for both the top and bottom figures. The inset for the bottom figure depicts the percentage values during the first hour after the administration of MP.

Scheme 1

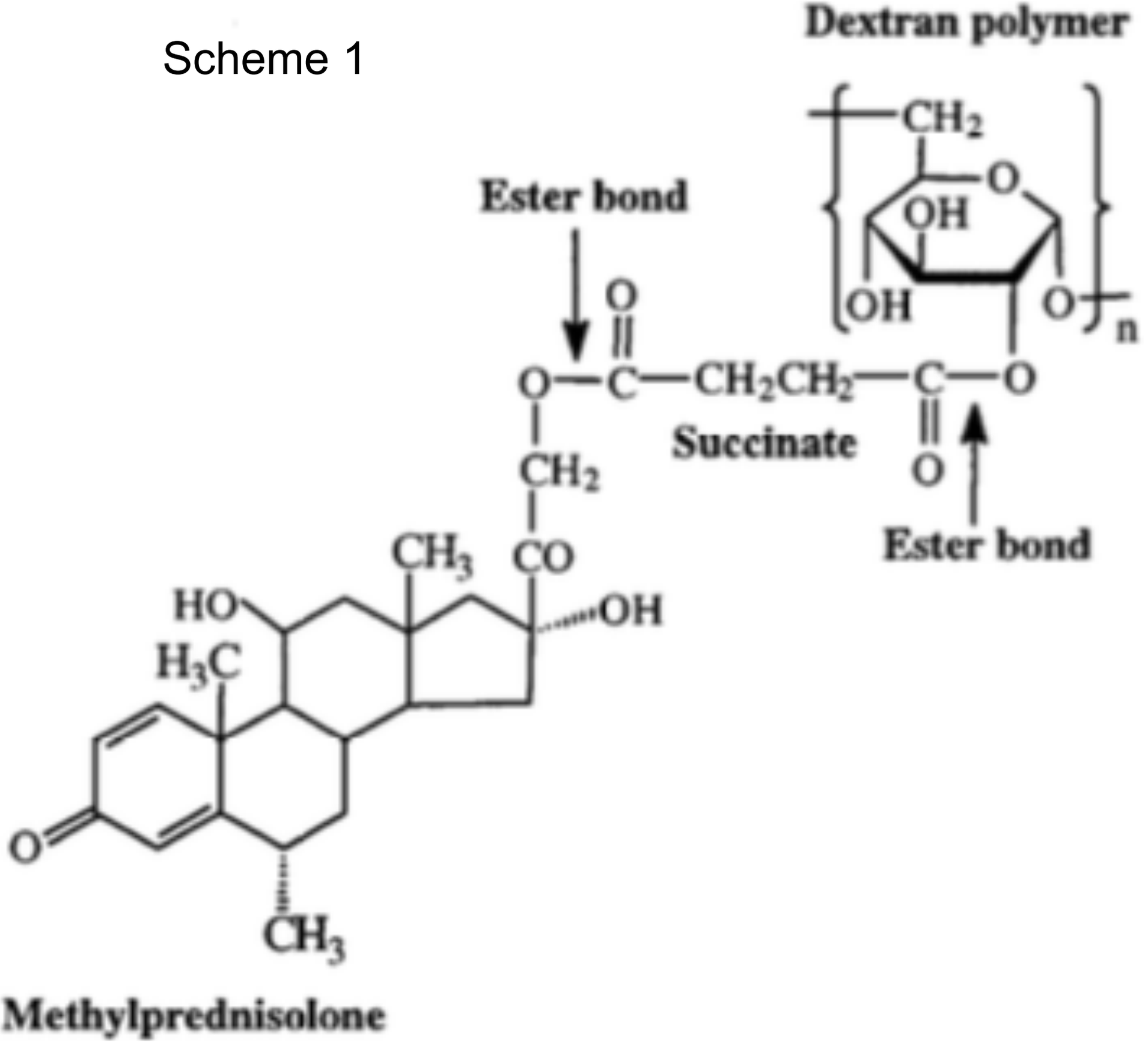

Dextran polymer
Ester bond
Succinate
Ester bond
Methylprednisolone

Figure 1

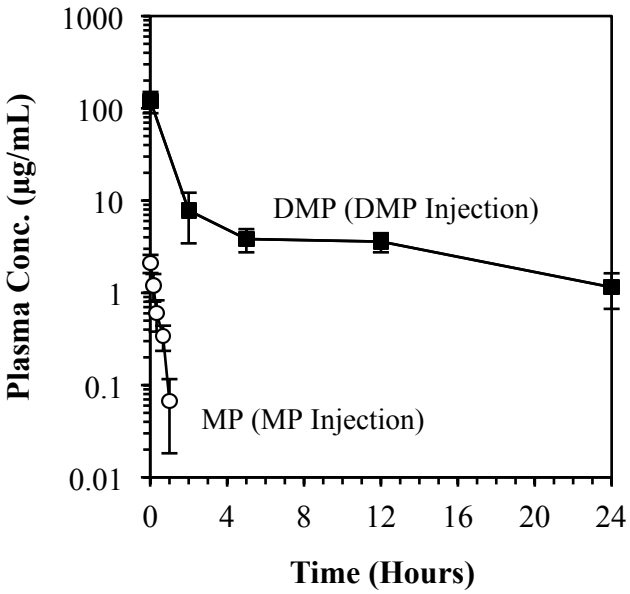

1000
100
10
1
0.1
0.01
Plasma Conc. (µg/mL)
DMP (DMP Injection)
MP (MP Injection)
0
4
8
12
16
20
24
Time (Hours)

Figure 2

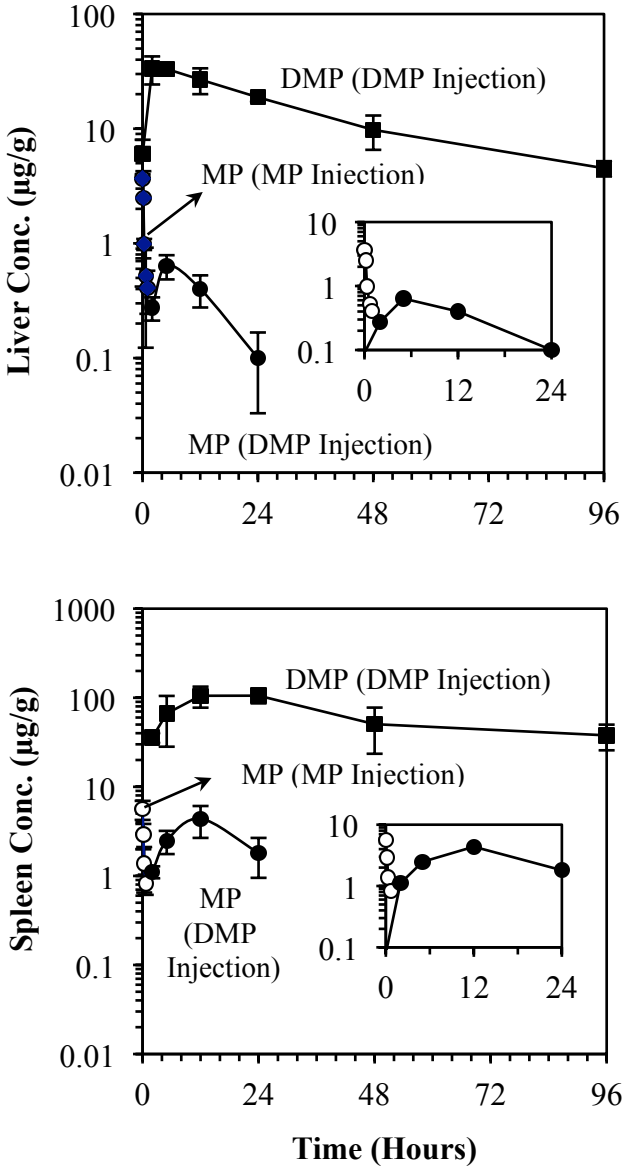
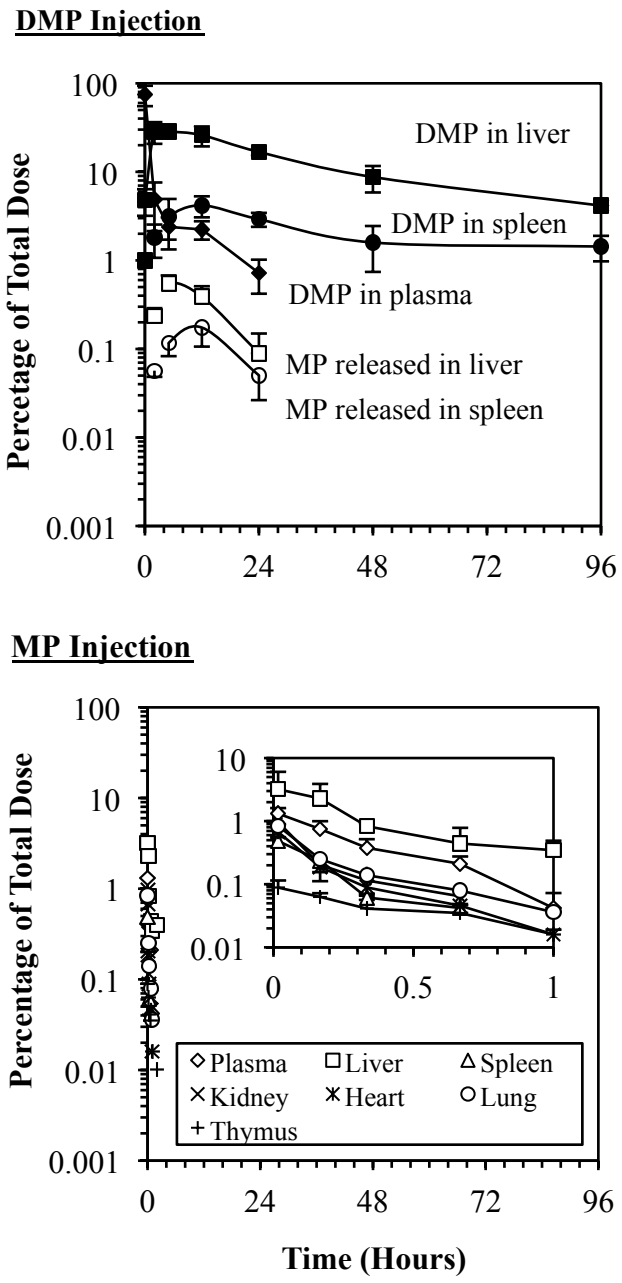

Liver Conc. (µg/g)
100
10
1
0.1
0.01
DMP (DMP Injection)
MP (MP Iniection)
MP (DMP Injection)
10
1
0.1
0
12
24
0
24
48
72
96
Spleen Conc. (µg/g)
1000
100
10
1
0.1
0.01
DMP (DMP Injection)
MP (MP Injection)
MP
(DMP
Injection)
10
1
0.1
0
12
24
0
24
48
72
96
Time (Hours)

Figure 3


DMP Injection
100
10
1
0.1
0.01
0.001
Percetage of Total Dose
0
24
48
72
96
DMP in liver
DMP in spleen
DMP in plasma
MP released in liver
MP released in spleen
MP Injection
100
10
1
0.1
0.01
0.001
Percentage of Total Dose
10
1
0.1
0.01
0
0.5
1
Plasma
Liver
Spleen
Kidney
Heart
Lung
Thymus
0
24
48
72
96
Time (Hours)