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The effect of SBE4-β-CD on i.v. methylprednisolone pharmacokinetics in rats: Comparison to a co-solvent solution and two water-soluble prodrugs

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Abstract

The i.v. pharmacokinetics of methylprednisolone (20 mg/kg) in six rats were studied after administration in a co-solvent (60:12:28 PEG 400/ethanol/water) mixture, a 0.075 M SBE4-β-CD solution (a sulfobutyl ether derivative variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin) and as its two water-soluble prodrugs, the 21-phosphate ester, disodium salt and the 21-hemisuccinate ester, monosodium salt. The aim of the work was to assess what effect the SBE4-β-CD would have on methylprednisolone pharmacokinetics while the comparison to the prodrugs would provide some insight into a formulation versus a chemical approach to the parenteral delivery of a sparingly water-soluble drug. The plasma concentration-time curves and the pharmacokinetic parameters of methylprednisolone from the SBE4-β-CD solution and co-solvent mixture were not significantly different. For example, the AUC ± S.E. values from zero to infinity of methylprednisolone from the co-solvent and the SBE4-β-CD solutions were 326.7 ± 20.6 and 317.4 ± 15.4 μg min ml^{-1}, respectively. The AUC values of methylprednisolone from its 21-phosphate and 21-hemisuccinate esters were 59.2 ± 4.4 and 33.17 ± 5.3% of that from the co-solvent, respectively. These results confirm that i.v. administered drugs such as methylprednisolone, appear to be rapidly and quantitatively released from SBE4-β-CD inclusion complexes. Modified cyclodextrins such as SBE4-β-CD may provide an alternative to the use of co-colvents, and possibly even prodrugs, for the parenteral delivery of sparingly water-soluble drugs such as methylprednisolone.

Keywords: Methylprednisolone; Pharmacokinetics; Prodrug; Phosphate ester; Hemisuccinate ester; Cyclodextrin, anionic; SBE4-β-CD; Sulfobutyl ether; Rat

1. Introduction

The objective of this work was to compare the intravenous (i.v.) pharmacokinetics of methylprednisolone in rats after its administration in a 60:12:28 polyethylene glycol 400 (PEG 400)/ethanol/water mixture, a 0.075 M SBE4-β-CD (a sulfobutyl ether derivative, sodium salt, variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin; Fig. 1; Rajewski, 1990; Stella and Rajewski, 1992) solution and as two water-soluble prodrugs, the 21-phosphate ester,

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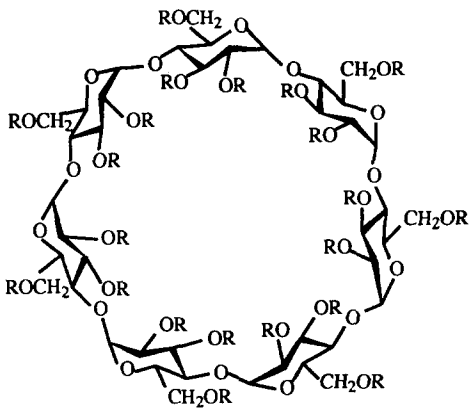

R = $-CH_2CH_2CH_2CH_2SO_3Na$ or -H
SBE4-β-CD
(average degree of sulfobutyl substitution is four)

Fig. 1. Schematic of the structure of SBE4-β-CD.

disodium salt and the 21-hemisuccinate ester, monosodium salt. The aim was to demonstrate that a parenterally safe (Rajewski, 1990), anionic cyclodextrin, SBE4-β-CD (Tait et al., 1992), would have a minimal effect on the pharmacokinetics of methylprednisolone compared to a co-solvent formulation. Comparison of the pharmacokinetics of the cyclodextrin formulation to the aqueous prodrugs would provide insight into a formulation versus a chemical approach to the delivery of a sparingly water-soluble drug like methylprednisolone.

Methylprednisolone is a synthetic glucocorticoid (Nelson and Dick, 1975) which is sparingly water soluble. For parenteral use, it is formulated as its water-soluble prodrug, the 21-hemisuccinate ester, monosodium salt. In dog studies, the systemic availability of methylprednisolone from its 21-hemisuccinate ester prodrug was found to be 43% (Toutain et al., 1986) and 59 ± 8.3% (Toutain et al., 1987) while in a rabbit study, it was found to be identical to that of methylprednisolone formulated in a Tween 80/ethanol mixture (Ebling et al., 1985). In rats, Kong and Jusko (1991) found that methylprednisolone systemic availability from its 21-hemisuccinate ester was 50–55% and 10% after intravenous and oral dosing, respectively.

Cyclodextrins have been extensively evaluated as a tool to improve the aqueous solubility of various drug molecules through the formation of inclusion complexes (Uekama et al., 1982; Brewster et al., 1989; Liu et al., 1990; Albers and Müller, 1992). However, the relatively low aqueous solubility of β-CD and its nephrotoxicity have precluded its use in parenteral dosage forms (Brewster et al., 1989). Efforts in these and other laboratories have been directed toward developing parenterally safe cyclodextrins. An anionic, modified β-CD, SBE4-β-CD is more water soluble than β-CD itself and has been identified as being safe after acute administration (Rajewski, 1990; Stella and Rajewski, 1992). The long-term parenteral safety of SBE7-β-CD, a material similar to SBE4-β-CD is being assessed.

Since it is expected that methylprednisolone would be released rapidly from its SBE4-β-CD inclusion complexes, the pharmacokinetics of methylprednisolone should not be affected by SBE4-β-CD solubilization. Few studies, however, have been published on the role of inclusion complexation by cyclodextrins on the pharmacokinetics of drugs after parenteral administration (Uekama et al., 1981, 1982, 1983; Arimori and Uekama, 1987; Brewster et al., 1989; Usayapant et al., 1991).

2. Experimental

2.1. Materials

Methylprednisolone and methylprednisolone 21-hemisuccinate, monosodium salt, were obtained from Sigma Chemical Co. (St. Louis, MO). Dexamethasone and PEG 400 were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methylprednisolone 21-phosphate, disodium salt, and methylprednisone were a gift from the Upjohn Co. (Kalamazoo, MI). SBE4-β-CD (average Mol. Wt 1708) was prepared in our laboratory using previously described methods (Rajewski, 1990; Stella and Rajewski, 1992). Male Sprague-Dawley

rats (six rats for each study) weighing 291 ± 42 g were used. Two types of cannulas were used in the experiments. A polyethylene tube (0.5 mm i.d., 0.8 mm o.d., Dural Plastics, Auburn, NSW, Australia) was beveled to a 45° angle for jugular vein cannulation and another polyethylene tube (1.4 mm i.d., 1.9 mm o.d., Clay-Adams, Parsippany, NJ) was also beveled to a 45° angle for tracheal cannulation.

2.2. Dosage form preparation

The methylprednisolone/co-solvent mixture dosage form was prepared by dissolving methylprednisolone (45–50 mg) in 3 ml of PEG 400, 0.6 ml of ethanol and sufficient water to make a final volume of 5 ml. Gentle heat (40°C) was used if needed to maintain the methylprednisolone in solution (Haughey and Jusko, 1992). This co-solvent cocktail and methodology was needed to maintain the methylprednisolone in solution for i.v. administration. The aqueous SBE4-β-CD dosage form was prepared by dissolving methylprednisolone (20–25 mg) in 5 ml of a 0.075 M SBE4-β-CD solution. Table 1 shows the effect of SBE4-β-CD (M) on the aqueous solubility (mg/ml) of methylprednisolone. A linear relationship was noted between methylprednisolone solubility and SBE4-β-CD concentration, indicating the formation of a 1:1 inclusion complex. The SBE4-β-CD solution (0.075 M) is slightly hypertonic. Two aqueous solutions of methylprednisolone prodrugs (methylprednisolone equivalent 7.5–8.3 mg/ml) were freshly prepared by dissolving the prodrugs in distilled de-ionized water. Tonicity was not adjusted.

Table 1
Aqueous solubility of methylprednisolone (mg/ml) in the presence of SBE4-β-CD (M)

SBE4-β-CD (M)	Methylprednisolone solubility (mg/ml)
0	0.078
0.011	0.95
0.027	2.09
0.066	5.09
0.11	7.70

2.3. Animal experimental protocol

The rats ($n = 6$ for each dosage form) were anesthetized by an intraperitoneal injection of sodium pentobarbital (50–55 mg/kg) shortly after mild ether anesthesia. Additional sodium pentobarbital injections were given whenever the animals showed signs of consciousness (4–5 times/3 h). A tracheotomy was performed to facilitate breathing. The left and right jugular veins were exposed and each vein was cannulated to administer the drug injection and obtain blood samples, respectively.

2.4. Drug administration and sampling

Intravenous administration of 20 mg/kg of methylprednisolone (or its equivalent for the prodrugs) in a 1 ml syringe was achieved over 30 s through the left jugular cannula. To avoid contamination, blood samples (about 200 μl) were taken from the right jugular vein at 15 min before and 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min after drug administration. Blood samples were transferred into pre-washed micro centrifuge tubes (Fisher Scientific micro centrifuge tubes, colorless, 1.5 ml) containing 50 μl of 0.07 M EDTA. The tubes were centrifuged and 50 μl of plasma was stored at −20°C until assayed.

2.5. Sample extraction and analysis

An internal standard spike, dexamethasone (250 ng/50 μl), was added to each plasma sample and 200 μl of methanol was added to precipitate plasma proteins. After centrifuging for 5 min (Fisher Scientific micro centrifuge, model 235C), the supernatant was collected, and the methanol was evaporated with a nitrogen stream (40°C). 200 μl of water were added to each sample and 500 μl of ethyl acetate were used to extract the drug and the internal standard. The samples were vortexed for 20 s and centrifuged for 5 min to separate the aqueous and organic layers. The ethyl acetate layer was decanted into a clean

micro centrifuge tube and the ethyl acetate extraction step was repeated. The combined extracts were evaporated under a nitrogen stream (40°C). The residue was reconstituted with 100 μl of mobile phase for HPLC analysis.

HPLC was performed using a system consisting of a Shimadzu LC-6A pump, a Shimadzu SPD-6A UV detector operating at 254 nm and a Shimadzu C-R3A integrator. Elution of each 20 μl sample was accomplished on a reversed phase ODS Hypersil column (15 cm × 4.6 mm, 5 μm particle size) using a mobile phase consisting of tetrahydrofuran/2-propanol/water (13.5:13.5:73) at a flow rate of 1.5 ml/min (Ebling et al., 1985). The quantitation limit for methylprednisolone from spiked plasma was 10 ng/ml. The standard curve for methylprednisolone from plasma was linear and the methylprednisolone peak was well resolved from interferences. The percent recovery of drug was calculated by measuring the peak area ratio of the standard internal standard and plasma internal standard. The average recovery of methylprednisolone from the plasma sample was 86.2 ± 5.6%.

2.6. Pharmacokinetic analysis

The pharmacokinetics of methylprednisolone from the co-solvent mixture and SBE4-β-CD were fitted using PC-NONLIN (Statistical Consultants Inc., Lexington, KY) to Eq. 1:

$$C = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \quad (1)$$

where C was the plasma concentration of methylprednisolone in μg/ml and time, t, was in min. The constants A_1, λ_1, A_2 and λ_2 were obtained from the computer fit of the experimental data to Eq. 1. The profiles of methylprednisolone from its two water-soluble prodrugs showed no apparent formation phase and could also be described by Eq. 1.

The area under the plasma concentration vs time curves, AUC(0–∞), the area under the moment curves, AUMC(0–∞), and clearance, D/AUC(0–∞), were calculated from the values of A_1, λ_1, A_2 and λ_2 for each animal. The absolute amount, or apparent bioavailability of methylprednisolone, F, was determined by comparing the AUC(0–∞) values for the SBE4-β-CD solution and the two prodrugs to the results from the co-solvent mixture (control). The mean residence time (MRT) of the drug in the body was calculated from Eq. 2:

$$\mathrm{MRT} = \mathrm{AUMC}(0-\infty)/\mathrm{AUC}(0-\infty) \quad (2)$$

The apparent elimination half-lives were calculated from the values of λ_2.

2.7. Statistical analysis

Statistical analyses were performed by using the computer program ANOVA (SV 512) to compare the data sets. In all cases, statistical significance was determined at the 95% confidence level ($p < 0.05$).

3. Results and discussion

The methylprednisolone co-solvent mixture dosage form was used as a control in these pharmacokinetic studies. Fig. 2 and 3 show the mean methylprednisolone plasma concentration vs time curves (with standard error bars, ±S.E.) for both

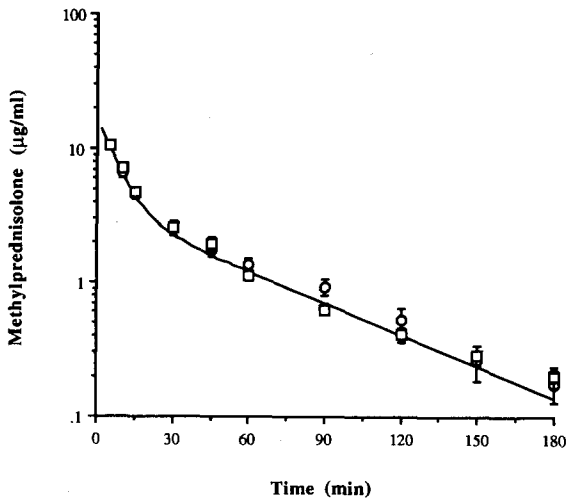

Fig. 2. Plasma concentration (±S.E.) vs time profile of methylprednisolone after a 20 mg/kg i.v. dose of methylprednisolone in anesthetized rats ($n = 6$) from a co-solvent mixture (○) and a 0.075 M aqueous SBE4-β-CD solution (□). The solid line is the curve fit for the co-solvent data to Eq. 1.

dosage forms and the two prodrugs, respectively, after i.v. administration (20 mg/kg) to rats. Methylprednisolone plasma concentration vs time profiles from the co-solvent and SBE4-β-CD solution can be defined by Eq. 3 and 4, respectively:

$$C = 14.1(\pm 1.7)e^{-0.139(\pm 0.028)t} + 3.5(\pm 0.3)e^{-0.018(\pm 0.003)t} \quad (3)$$

$$C = 12.8(\pm 1.1)e^{-0.100(\pm 0.012)t} + 2.6(\pm 0.1)e^{-0.016(\pm 0.002)t} \quad (4)$$

There were no significant differences in any of the fitted parameters (A_1, λ_1, A_2 and λ_2) between the two dosage forms. The mean value for the half-life of methylprednisolone from the co-solvent was 41.5 ± 3.6 min. This mean value is a little longer than those reported by Kong and Jusko (1991) of 24.9 to 27.8 min in rats administered i.v. 10 and 50 mg/kg methylprednisolone in the same co-solvent vehicle, respectively. However, in the aforementioned study, the animals were not anesthetized. Similar values for the half-lives were seen from the other dosage forms. A longer half-life is not unexpected under anesthetic conditions. The plasma concentrations of methylprednisolone from the two prodrugs were significantly lower than those from the co-solvent and the SBE4-β-CD formulations (Fig. 3).

Some mean pharmacokinetic parameters are listed in Table 2. The AUC(0–∞)values of methylprednisolone from SBE4-β-CD and the co-solvent, calculated by integration of the fitted curves, were not significantly different. The AUC values to 180 min calculated by linear trapezoidal rule or the Lagrange method were similar to those from the integrated values and, again, were also not significantly different. AUMC(0–∞) values followed the trend for the AUC(0–∞) values but MRT values were identical across the dosage forms.

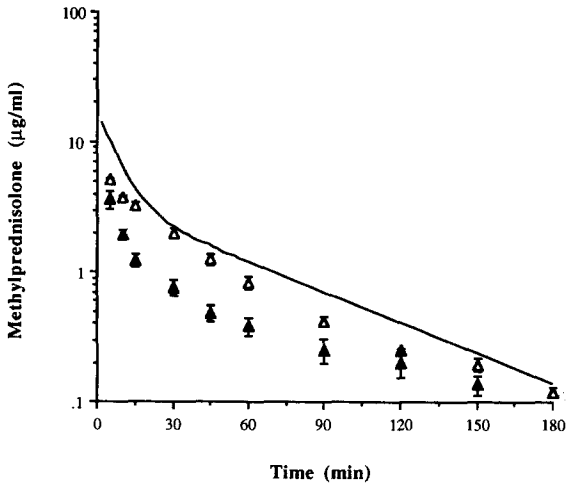

Fig. 3. Plasma concentration (±S.E.) vs time profile of methylprednisolone after a 20 mg/kg i.v. dose of methylprednisolone (or its equivalent) in anesthetized rats ($n = 6$) from its 21-phosphate prodrug (△) and its 21-hemisuccinate prodrug (▲). The solid line is that for methylprednisolone from the co-solvent solution (see Fig. 2).

The absolute bioavailability of methylprednisolone was determined by dividing the AUC(0–∞) values of methylprednisolone from the SBE4-β-CD, the 21-phosphate and 21-hemisuccinate prodrug solutions into the co-solvent control AUC(0–∞) values. One of the aims of the present study was to determine the bioavailability of methylprednisolone from the SBE4-β-CD solu-

Table 2
Average methylprednisolone pharmacokinetic parameters (±S.E.) for four different dosage forms after i.v. administration (20 mg/kg) to anesthetized rats ($n = 6$)

Dosage form	$t_{1/2}$ (min)	AUC(0–∞) (μg min ml^{-1})	Clearance (ml min^{-1} kg^{-1})	AUMC(0–∞) (μg min^2 ml^{-1}) (×10^{-3})	MRT (min)	F (%) [a]
Co-solvent	41.5 ± 3.6	326.7 ± 20.6	62.7 ± 4.7	14.9 ± 2.5	42.0 ± 7.3	100
SBE4-β-CD	43.2 ± 2.0	317.4 ± 15.4	63.8 ± 3.1	13.2 ± 1.1	41.5 ± 2.1	97.1 ± 4.7
21-Phosphate	45.2 ± 4.3	199.2 ± 13.9 [b]	–	9.9 ± 1.6	48.2 ± 4.0	59.2 ± 4.4 [b]
21-Hemisuccinate	40.6 ± 13.5	108.4 ± 17.3 [b]	–	7.0 ± 2.7 [b]	55.5 ± 13.6	33.2 ± 5.3 [b]

[a] Systemic availability.
[b] Significantly different from co-solvent control ($p < 0.05$).

tion and its two prodrugs compared to the co-solvent solution. As shown in Table 2, the apparent bioavailability of methylprednisolone from the SBE4-β-CD solution was not significantly different from that of the co-solvent. This, along with other pharmacokinetic parameter values strongly suggest that the methylprednisolone is rapidly and quantitatively released from the inclusion complex.

Methylprednisolone plasma concentrations following administration of its 21-phosphate and 21-hemisuccinate esters indicate less than quantitative release since the relative AUC values gave bioavailabilities of 59.2 ± 4.4 and 33.2 ± 5.3%, respectively. As in previous studies, the 21-phosphate ester released methylprednisolone more completely than the 21-hemisuccinate ester (Möllmann et al., 1988). Others (Narang et al., 1983) have assumed that this is due to the more rapid in vivo cleavage of the 21-phosphate ester prodrug relative to the 21-hemisuccinate ester. While the bioavailability of methylprednisolone from its 21-hemisuccinate ester in rabbits was found to be quantitative (Ebling et al., 1985) its bioavailability from its 21-hemisuccinate in dogs was estimated to be 43% (Toutain et al., 1986). This low value was explained as being due to sequential metabolism of methylprednisolone to other metabolites (Toutain et al., 1986). In a human study, the 21-hemisuccinate ester prodrug was found to have 71% availability compared to the 21-phosphate ester prodrug (Möllmann et al., 1988). In rats, the incomplete availability of methylprednisolone from its 21-phosphate and 21-hemisuccinate esters can be explained by the two hypotheses that its systemic availability is affected by either a sequential single-pass hepatic metabolism or by elimination prior to their conversion to methylprednisolone. The very high clearance of methylprednisolone seen in the present study (Table 2) and that observed by Kong and Jusko (1991) is more consistent with the first hypothesis. The lack of an apparent formation phase of methylprednisolone seen with the prodrugs is also more consistent with the sequential first-pass hypothesis.

Methylprednisolone is reversibly metabolized to methylprednisone in most animal species. Although an attempt was also made to analyze methylprednisone in the plasma samples, an unexpected interfering peak prevented quantitation in some rats. For those rats where methylprednisone could be quantitated with some confidence, the following AUC (trapezoid) values were estimated; 55.9 ± 3.8 μg min ml^{-1} ($n = 3$, co-solvent); 47.3 ± 5.4 μg min ml^{-1} ($n = 3$, SBE4-β-CD); 15.6–23.9 μg min ml^{-1} (range for $n = 2$, 21-phosphate); 1.7–3.2 μg min ml^{-1} (range for $n = 2$, 21-hemisuccinate). Qualitatively, the relative levels of methylprednisone appeared to follow the same rank order seen in the AUC values for methylprednisolone from the two formulations and the prodrugs. These observations confirm the formation of methylprednisone from methylprednisolone and the order of availability from the various dosage forms and prodrugs.

4. Summary

Methylprednisolone is rapidly and quantitatively released from an SBE4-β-CD aqueous solution compared to a co-solvent control in anesthetized rats. Although β-cyclodextrin has both limited aqueous solubility and has been shown to be nephrotoxic (Irie et al., 1982; Pitha et al., 1986), SBE4-β-CD is more water soluble and did not show any toxicity in mice after parenteral administration (Rajewski, 1990; Stella and Rajewski, 1992). Providing the safety of SBE4-β-CD and SBE7-β-CD (the most probable clinical material) can be confirmed, they may be useful alternatives to the use of co-solvents and possibly even prodrugs, for the parenteral delivery of sparingly water-soluble drugs like methylprednisolone.

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