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The effect of SBE4-β-CD on i.m. prednisolone pharmacokinetics and tissue damage in rabbits: Comparison to a co-solvent solution and a water-soluble prodrug

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Abstract

The i.m. pharmacokinetics of prednisolone (5 mg/kg) in eight rabbits after its administration in a co-solvent (40:10:50; PEG 400/ethanol/water) mixture, in a slightly hypertonic 0.09 M SBE4-β-CD (a sulfobutyl ether derivative variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin) solution and from a water-soluble prodrug, the 21-phosphate ester, disodium salt were studied. Muscle damage as measured by changes in plasma creatine kinase (CK) levels caused by the administration of the three solutions was also assessed. The prednisolone plasma AUC values over 24 h from the SBE4-β-CD formulation and the phosphate ester were 87.0 ± 12.6 and 78.0 ± 14.1% of that from co-solvent, respectively. The apparent bioavailability of prednisolone over 24 h from the SBE4-β-CD formulation and its prodrug was not significantly different from that of the co-solvent. The changes in CK levels from the SBE4-β-CD were identical to those from normal saline, however, the co-solvent mixture caused significantly elevated CK levels. The presence or absence of prednisolone had no effect on the relative CK levels for the cyclodextrin solution and the normal saline. There was a small effect noted for the co-solvent, with and without prednisolone. These results confirm that i.m. administered drugs, such as prednisolone, appear to be rapidly, quantitatively and safely released from SBE4-β-CD inclusion complexes. SBE4-β-CD may provide an alternative to the use of co-colvents and possibly even prodrugs for the i.m. delivery of sparingly water-soluble drugs such as prednisolone.

Keywords: Prednisolone; Pharmacokinetics; Prodrug; Phosphate ester; Cyclodextrin, anionic; SBE4-β-CD; Sulfobutyl ether; Rabbit

1. Introduction

The objective of this work was to compare the muscle damage, as measured by the elevation of the intracellular enzyme, creatine kinase (CK), in plasma and the intramuscular pharmacokinetics of prednisolone in rabbits after its administration in a 40:10:50 polyethylene glycol 400 (PEG 400)/ethanol/water mixture, a 0.09 M SBE4-β-CD (a sulfobutyl ether derivative variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin; Rajewski, 1990; Stella and Rajewski,

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1992; Stella et al., 1995) solution and as a water-soluble prednisolone prodrug, the 21-phosphate ester, disodium salt. The aims were to demonstrate that a parenterally safe, anionic cyclodextrin, SBE4-β-CD (Tait et al., 1992), would have a minimal effect on the i.m. pharmacokinetics of prednisolone compared to a co-solvent formulation and to assess whether it caused any significant local tissue damage. Comparison to the prodrug would provide insight into a formulation versus a chemical approach to the i.m. delivery of a sparingly water-soluble drug like prednisolone. A rabbit model was chosen over a rodent model because of the larger muscle mass of rabbits (Kaplan and Timmons, 1979) and because it appears to be the acceptable model for muscle irritancy studies.

Prednisolone is sparingly water soluble (Table 1). The major, immediate release form of prednisolone for i.v. or i.m. administration is the water-soluble 21-phosphate ester prodrug (Pickup et al., 1977; Frey and Frey, 1990). The release of prednisolone from its 21-phosphate has not been studied extensively (Musson et al., 1991), although the release of other steroids from their 21-phosphate esters has been well documented (Loo et al., 1981; Miyabo et al., 1981; Rohdewald et al., 1987; Möllmann et al., 1988, 1989; Stella et al., 1995).

Prednisolone can also be solubilized by the use of organic co-solvents. However, i.m. administration of organic co-solvents can cause muscle damage (Brazeau and Fung, 1989a,b, 1990a,b). The mechanism of muscle damage caused by organic co-solvents has not been clearly explained. Brazeau and Fung (1989c) showed that co-solvent induced creatine kinase release from rat muscle was not correlated with the physicochemical properties of the aqueous co-solvent mixtures, i.e., tonicity, dielectric constant, pH, viscosity or surface tension. A possible mechanism for co-solvent induced myotoxicity is suggested to be the biochemical interaction between organic co-solvents and muscle fibers (Brazeau and Fung, 1990b). These organic co-solvents have also been shown to cause hemolysis (Fort et al., 1984; Reed and Yalkowsky, 1985).

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). 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; Stella et al., 1995). The long-term parenteral safety of SBE7-β-CD, a material similar to SBE4-β-CD is under extensive evaluation.

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

SBE4-β-CD (M)	Prednisolone solubility (mg/ml)
0	0.27
0.025	7.3
0.055	13.6
0.11	26.6

2. Experimental

2.1. Materials

Prednisolone, prednisone and a commercial creatine kinase reagent kit were purchased from Sigma Chemical Co. (St. Louis, MO). Prednisolone 21-phosphate, disodium salt was obtained from Steroidals, Inc. (Wilton, NH). Dexamethasone and PEG 400 were obtained from Aldrich Chemical Co. (Milwaukee, WI) while SBE4-β-CD was prepared in our laboratory. Normal saline was obtained from Baxter Healthcare Co. (Deerfield, IL). Eight male New Zealand

White rabbits weighing 4.4 ± 0.3 kg were used with a 2 week washout period between studies.

2.2. Dosage form preparation

The co-solvent dosage form of prednisolone was prepared by dissolving prednisolone (20–23 mg) in 0.8 ml of PEG 400, 0.2 ml of ethanol and adding water to make a final volume of 2 ml (40:10:50). For the SBE4-β-CD dosage form, prednisolone (20–23 mg) was dissolved in 2 ml of a 0.09 M SBE4-β-CD solution (see Table 1). The SBE4-β-CD solution (0.09 M) is slightly hypertonic. An aqueous isotonic prednisolone 21-phosphate ester, disodium salt (prednisolone equivalent 20–23 mg) dosage form was prepared by dissolving the prodrug in a 0.68% sodium chloride solution to maintain tonicity. The blank solutions for the muscle damage studies were the co-solvent mixture, 0.09 M SBE4-β-CD solution and normal saline.

2.3. Animal experiment protocol

Eight Male New Zealand White rabbits were used in two, three-way randomized cross-over studies, the first, to measure CK release from the blank vehicles after i.m. injection and the second, to measure prednisolone and CK release after i.m. injection from the chosen dosage form. The rabbits were gradually familiarized with the restrainers and investigators before the experiments. A 23-gauge 1-inch needle and 3 ml syringe were used for the intramuscular injection study, and a 26-gauge 3/8-inch needle and 1 ml syringe were used for blood sampling. To avoid injecting too much solution volume in one injection site, 1 ml was injected into the right mid-lumbar muscle and 1 ml injected into the left mid-lumbar muscle for both the bioavailability and muscle damage studies. To ensure i.m. administration, the needle was inserted into the muscle at an angle perpendicular to the skin for the full 1 inch of the needle. Blood samples were obtained from the right and left marginal ear veins alternatively with direct needle puncture.

2.4. Muscle damage study

Muscle damage caused by the blank solutions was determined by measuring the plasma CK levels after each i.m. injection of 2 ml of three drug-free solutions. Blood samples (100 μl) were obtained at 15 min before injection and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the i.m. injection. Blood samples were transferred into heparinized micro centrifuge tubes (Fischer Scientific micro centrifuge tubes, colorless, 1.5 ml), centrifuged and 50 μl of plasma was stored at −20°C until assayed. Plasma CK levels were analyzed using a commercial CK Reagent kit. After the mixing and sample incubation, changes in absorbance over a 2 min period at 340 nm were read and recorded on a Shimadzu UV 260 UV-Visible Recording Spectrophotometer.

2.5. Prednisolone bioavailability study

Three dosage forms of prednisolone (5 mg/kg) were injected into both the left and right mid-lumbar muscles using the same needle and syringe size as for the CK study. Blood samples (1 ml) were collected in heparinized micro-centrifuge tubes at 15 min before injection and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h of after dosing and 400 μl of plasma was stored at −20°C until assayed. Dexamethasone was used as an internal standard, and 500 μl of ethyl acetate was added to the plasma sample with the internal standard (250 ng). The samples were vortexed for 20 s and centrifuged for 5 min to separate the aqueous and organic phases. The organic phase was decanted into a clean micro centrifuge tube. The extraction step was repeated in order to improve the recovery. The combined organic phases were evaporated under a nitrogen stream (40°C) and the residue was reconstituted with 100 μl of mobile phase for HPLC analysis.

HPLC was performed using the system described in the preceding paper for methylprednisolone (Stella et al., 1995) except that the mobile phase consisted of tetrahydrofuran/methanol/water (25:12.5:62.5) at a flow rate of 1.8 ml/min. The drug concentration was determined by comparison to a plasma prednisolone standard

curve which was linear over the range of plasma concentrations needed. The prednisolone quantitation limit was 10 ng/ml. The average recovery compared to a spiked plasma standard was 92.6 ± 2.4%. Creatine kinase levels were also measured after the i.m. injection of drug solution. Analyses for CK levels were the same as for the blank study process.

2.6. Pharmacokinetic data analysis

The area under the plasma concentration vs time curve, AUC(0–24 h), was calculated by both the linear trapezoidal and the Lagrange method (Yeh and Kwan, 1978). The relative bioavailability of prednisolone from the SBE4-β-CD dosage form and from its 21-phosphate ester prodrug were determined by comparison to the results from the co-solvent mixture (control). The percent bioavailability, F (%), was determined according to Eq. 1:

$$F(\%) = \mathrm{AUC}(0-\infty)_x / \mathrm{AUC}(0-\infty)_{\text{co-solvent}} \times 100 \quad (1)$$

where $\mathrm{AUC}(0-\infty)_x$ is the AUC from the SBE4-β-CD dosage form or a molar equivalent of the 21-phosphate ester, and $\mathrm{AUC}(0-\infty)_{\text{co-solvent}}$ denotes the AUC of prednisolone from the co-solvent formulation. Since no measurable prednisolone levels were seen at 24 h post-dosing, AUC values to 24 h were assumed to be equivalent to those to infinity. C_{max} and T_{max} were the average values of the maximum plasma concentration and time, respectively. Estimates of the apparent biological half-life for prednisolone were made from log-linear regression analysis of the 3–12 h plasma samples.

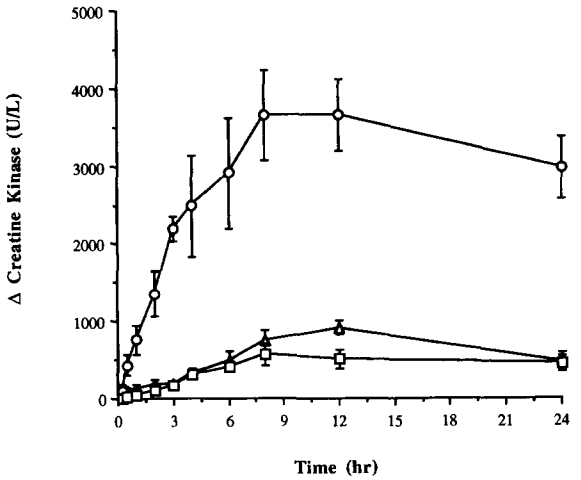

Fig. 1. Change in plasma CK levels (±S.E.) after i.m. injection of 1 ml into both right and left lumbar muscles of a PEG 400/ethanol/water (40:10:50) mixture (○), a 0.09 M SBE4-β-CD solution (□) and normal saline (△) to rabbits ($n = 8$).

2.7. Statistical analysis

Statistical analysis of the various data sets was performed using the computer program ANOVA (SV 512). In all cases statistical significance was determined at the 95% confidence level ($p < 0.05$).

3. Results and discussion

Propylene glycol, ethanol and polyethylene glycol are the water-miscible organic co-solvents most frequently used to solubilize drugs. The co-solvent mixture of PEG 400 (40%), ethanol (10%) and water to 100% is similar to those evaluated by others (Reed and Yalkowsky, 1985; Brazeau and Fung, 1989b). In the present study, such a solution was needed to sufficiently solubilize 22–23 mg of prednisolone. When a phase solubility study of prednisolone in aqueous SBE4-β-CD solutions was performed, a 0.09 M SBE4-β-CD solution was capable of dissolving the same quantity of prednisolone. Since a plot of prednisolone solubility vs SBE4-β-CD was linear, a 1:1 inclusion complex was assumed to be the mode of solubilization (see Table 1).

3.1. Muscle damage from prednisolone-free solutions

The change in plasma CK level vs time curves are shown in Fig. 1 for the three blank vehicles. The CK levels were corrected by subtracting the baseline CK activity determined from a plasma sample obtained 15 min before dosing. The corrected CK, area under the curve, CK-AUC(0–24

h), was calculated by the linear trapezoidal method. The CK-AUC(0–24 h) for the aqueous co-solvent mixture was $7.9 \pm 1.1 \times 10^4$ U h l^{-1}. This was significantly higher than the CK-AUC(0–24 h) values for the 0.09 M SBE4-β-CD solution and normal saline, which were $1.1 \pm 0.3 \times 10^4$ and $1.4 \pm 0.2 \times 10^4$ U h l^{-1}, respectively. The CK-AUC(0–24 h) values for the SBE4-β-CD solution and normal saline were not significantly different from each other.

Both the normal saline and the 0.09 M SBE4-β-CD solution caused a small increase in the CK levels over the baseline levels. Therefore, a preliminary study was performed with blood sampling without i.m. injection of any solution to determine if the slight elevation in the CK levels seen with the normal saline and the SBE4-β-CD solution could be due tissue damage caused by the multiple venous punctures. The results for a number of rabbits and different plasma sample numbers are shown in Fig. 2. The more plasma samples taken the greater the elevation in plasma CK levels. It appears as if the elevation seen with the normal saline and the SBE4-β-CD solution may be attributable to the tissue damage caused by the venous punctures. These results are consistent with earlier observations by Hsu and Watanabe (1983). The results suggest that the 0.09 M SBE4-β-CD solution caused minimal, if any, tissue damage.

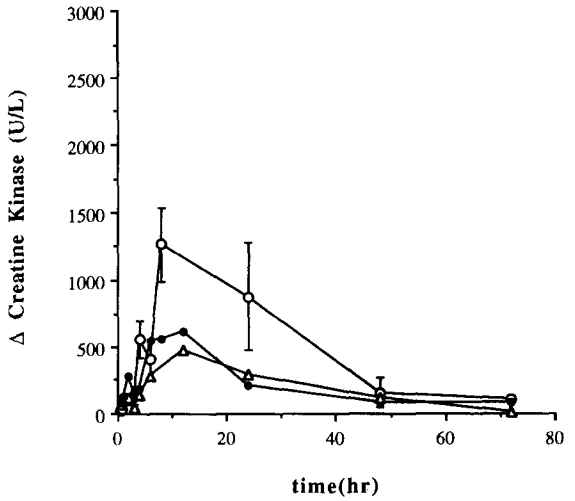

Fig. 2. Changes in plasma CK levels (±S.E. where more than one animal was used) after multiple blood sampling at indicated times from the right and left marginal ear veins of rabbits; eight sample times (same as final sampling schedule, △, $n = 1$); 11 sample times (●, $n = 1$); 17 sample times (○, $n = 3$).

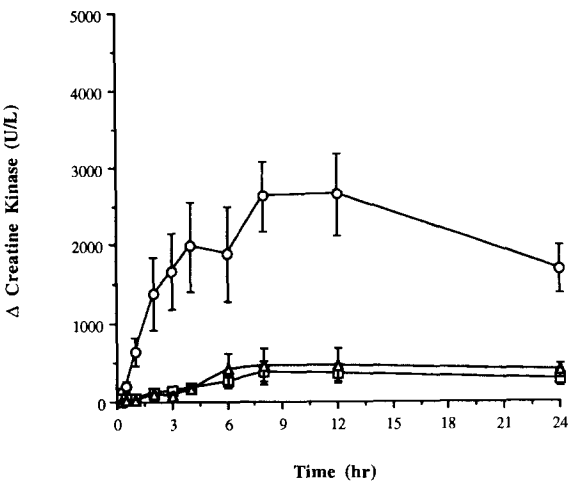

Fig. 3. Change in plasma CK levels (±S.E.) after i.m. injection of 1 ml into both right and left lumbar muscles of prednisolone in a PEG 400/ethanol/water (40:10:50) mixture (○), a 0.09 M SBE4-β-CD solution (□) and from an isotonic prednisolone 21-phosphate solution (△) to rabbits ($n = 8$).

3.2. Muscle damage from prednisolone containing solutions

The changes in plasma creatine kinase levels after i.m. administration of the three prednisolone containing dosage forms are demonstrated in Fig. 3. The corrected change in CK-AUC(0–24 h) from the co-solvent, 0.09 M SBE4-β-CD solution and the 21-phosphate ester prodrug solution were $5.1 \pm 1.0 \times 10^4$, $7.7 \pm 2.3 \times 10^3$ and $9.7 \pm 3.3 \times 10^3$ U h l^{-1}, respectively. Like the results from the prednisolone free formulations, the co-solvent mixture caused significantly higher CK release when compared to the SBE4-β-CD and the isotonic 21-phosphate prodrug solutions.

There were no significant differences between the CK-AUC values, with and without prednisolone, for the saline/prodrug formulations and the two SBE4-β-CD solutions. There was a signif-

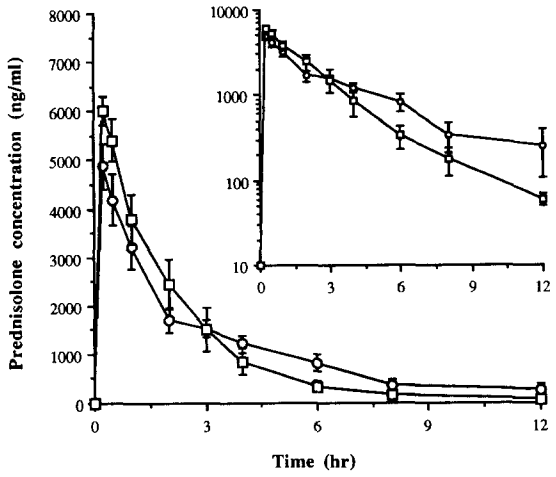

Fig. 4. Concentration (±S.E.) vs time profiles for prednisolone after 5 mg/kg i.m. injection of a 1 ml solution into both the right and left lumbar muscles of rabbits ($n = 8$) from a co-solvent mixture (○) and a 0.09 M SBE4-β-CD solution (□). Inset is the same data plotted on semilog scale.

icant decrease in the AUC with the prednisolone in the co-solvent compared to the co-solvent blank. This may have been due to the local anti-inflammatory action of the prednisolone.

3.3. Bioavailability of prednisolone from i.m. injection

The prednisolone co-solvent mixture dosage form was used as a control for the bioavailability studies. Fig. 4 and 5 show the mean prednisolone concentration (±S.E.) vs time curves for both the co-solvent and SBE4-β-CD solutions and from the co-solvent and the prodrug solutions, respectively. The mean pharmacokinetic parameters determined for each dosage form are listed in Table 2.

Prednisolone is reversibly metabolized to prednisone in most animal species. Although an attempt was also made to analyze prednisone in the plasma samples, an unexpected interfering peak prevented quantitation in some rabbits. For those rabbits where prednisone could be quantitated with some confidence, the following AUC (trapezoid) values were estimated; 0.76 ± 0.34 μg min ml^{-1} ($n = 3$, co-solvent); 0.94 ± 0.19 μg min ml^{-1} ($n = 3$, SBE4-β-CD); 0.15 ± 0.04 μg min ml^{-1} ($n = 3$, 21-phosphate). No attempt was made to analyze the prednisolone/prednisone data by a more sophisticated model proposed by Huang and Jusko (1989), since our principle goal was to determine whether SBE4-β-CD grossly altered the pharmacokinetics of prednisolone after i.m. dosing.

From the relative prednisolone AUC values calculated by the Lagrange method, the percentage bioavailability of prednisolone from the SBE4-β-CD formulation was $87.0 \pm 12.6\%$ which was not significantly different from 100%, i.e., prednisolone appeared to be quantitatively released from the SBE4-β-CD inclusion complex after i.m. injection. Prednisolone AUC values from the 21-phosphate ester prodrug were also not significantly lower than those from the co-solvent control and from the SBE4-β-CD solution. When the AUC values and relative bioavailabilities were assessed using a simple trapezoid method, the results were essentially identical except that there was a statistically significant decrease in the relative bioavailability of pred-

Table 2
Average pharmacokinetic parameters (±S.E.; $n = 8$) for prednisolone (5 mg/kg or its equivalent for the 21-phosphate prodrug) after i.m. injection of 1 ml into the right and left lumber muscles of rabbits from a co-solvent mixture, a 0.09 M SBE4-β-CD solution and from a prednisolone 21-phosphate ester solution

Dosage form	AUC(0–∞) (μg h ml^{-1})	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/ml)	F (%) [a]
Co-solvent	14.6 ± 2.8	2.5 ± 0.9	0.41 ± 0.09	4.58 ± 0.59	100
SBE4-β-CD	10.5 ± 0.6	1.5 ± 0.2	0.47 ± 0.12	6.45 ± 0.35 [b]	87.0 ± 12.6
21-Phosphate	10.0 ± 0.9	1.6 ± 0.1	0.32 ± 0.04	6.16 ± 0.65 [b]	78.0 ± 14.1

[a] Bioavailability, $AUC(0-\infty)_{dosage\ form}/AUC(0-\infty)_{co\text{-}solvent} \times 100$.
[b] Significantly different ($p < 0.05$) from the co-solvent control.

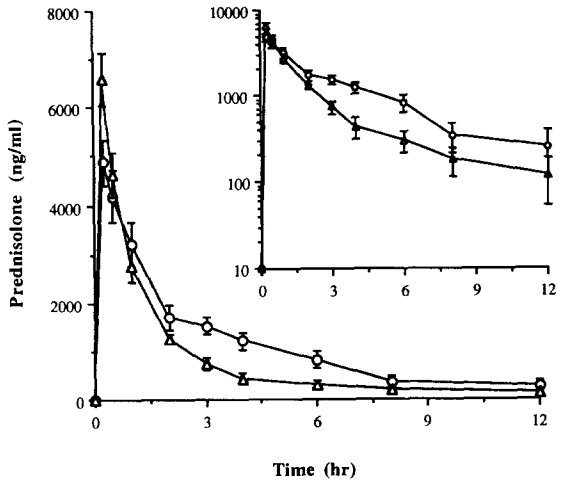

Fig. 5. Concentration (±S.E.) vs time profile of prednisolone after a 5 mg/kg i.m. injection of 1 ml solutions into both the right and left lumbar muscles of rabbits ($n = 8$) from a co-solvent mixture (○) and from an isotonic solution of prednisolone 21-phosphate, disodium salt (△). Inset is the same data plotted on a semilog scale.

nisolone from the 21-phosphate ester relative to the co-solvent control.

The loss of plasma prednisolone appears to follow first-order kinetics (inset, Fig. 4), with an apparent biological half-life of 1.5 h (Table 2). The complete release, relative to the co-solvent formulation, the early T_{max} and high C_{max} values are consistent with rapid and quantitative release of prednisolone from the injection site. However, the longer apparent half-life for prednisolone from the co-solvent (Table 2 and inset, Fig. 4) and the significantly lower C_{max} value, but an early T_{max} value suggest that a significant portion of the prednisolone dose is rapidly released from the site but that a portion of the dose may also have precipitated. Slow dissolution of the precipitated material could account for the longer apparent prednisolone half-life from the co-solvent. This phenomenon has also been observed by others for drugs administered in co-solvents, e.g., diazepam and digoxin, where the drug precipitates due to a pH difference between the formulation and physiological pH values, or a combination of co-solvent dilution and pH shift (Boxenbaum et al., 1977).

4. Summary

The degree of muscle damage caused by an i.m., slightly hypertonic SBE4-β-CD injection was significantly less than the co-solvent control and was similar to that observed for a normal saline injection. Intramuscular administered prednisolone appears to be rapidly, quantitatively and safely released from SBE4-β-CD inclusion complexes. Therefore, SBE4-β-CD may provide an alternative to the use of co-colvents and possibly even prodrugs for the i.m. delivery of sparingly water-soluble drugs such as prednisolone, providing the long-term safety of SBE4-β-CD and SBE7-β-CD (the most probable clinical material) can be confirmed.

Acknowledgements

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