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Absorption and tissue tolerance of ricobendazole in the presence of hydroxypropyl-β-cyclodextrin following subcutaneous injection in sheep

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ABSTRACT

Post-injection precipitation may cause poor and erratic drug absorption and tissue irritation at the injection site. Tissue tolerance and pharmacokinetics of a low pH ricobendazole (RBZ) injectable containing 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) were simultaneously investigated after subcutaneous injection in sheep compared to a reference formulation without HP-β-CD. Each animal received a RBZ containing formulation on one side of the back and the respective vehicle on the contralateral side. The HP-β-CD vehicle showed good tissue tolerance and the acidic solution caused minimal injection site reactions. Both RBZ containing formulations caused pain on injection and tissue histological changes in some animals. Lack of elevation of plasma creatine kinase indicated that none of the formulations caused significant damage to the underlying muscle tissue. Compared to the reference formulation, AUC and C_{max} of the HP-β-CD formulation were 1.6 and 2.2 times higher, respectively, whereas t_{max}, MRT and $t_{1/2}$ were significantly shorter suggesting faster and greater absorption of RBZ in the presence of HP-β-CD. This was attributed to the effect of inhibition of post-injection drug precipitation and drug absorption enhancement of HP-β-CD. In conclusion, HP-β-CD was shown to be a tissue-compatible excipient with potential to inhibit post-injection precipitation and increase absorption of poorly water soluble drugs. Additionally, the HP-β-CD formulation showed promise as an injectable that potentially minimizes irritation by reducing the dose required.

1. Introduction

Irritation and post-injection precipitation are concerns in parenteral drug delivery for poorly water soluble drugs. It has been estimated that about 10% of the drugs on the market have solubility problems (Muller et al., 2004), and an estimated 40% of agents in development are poorly water soluble (Lipinski, 2002).

A number of solubilization approaches have been investigated for parenteral formulations. The most commonly used approaches include (1) pH adjustment or salt formation; (2) addition of organic co-solvents and (3) formation of micelles using surfactants. However, concerns associated with these solubilization approaches are the risk of tissue damage at the injection site (Brazeau and Fung, 1989, 1990; Brazeau et al., 1998) and post-injection drug precipitation (Tuttle, 1977; Simamora et al., 1996; Yalkowsky et al., 1998). Post-injection drug precipitation may cause problems in several ways: (1) mechanical irritation, or thrombosis caused by the particles of the precipitated drug; (2) irritation to the tissues at the injection site due to the prolonged drug-tissue contact time; and (3) the likelihood of poor and less reproducible systemic bioavailability, which has been summarized in the literature (Tuttle, 1977).

Complexation with cyclodextrins, particularly with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulphobutylether-β-cyclodextrin (SBE-β-CD), has been intensively investigated as a solubilization approach in parenteral formulations. Cyclodextrins are cyclic oligosaccharides consisting of covalently linked glucopyranose rings in a coronal or cone shape. The inclusion complex is formed when a non-polar 'guest' molecule is partially or fully included inside a 'host', e.g. cyclodextrin molecule. Formulations of these complexes have been shown to be less irritant compared to the drug alone (Nagase et al., 2002, 2003) or the co-solvent formulations (Doenicke et al., 1994; Stella et al., 1995; Medlicott et al., 1998). In addition, cyclodextrin formulations of poorly water soluble drugs showed little or no tendency for drug precipitation after intramuscular injection (Irie and Uekama, 1997; Stella and He, 2008). Currently, there are at least six injectable products using HP-β-CD (Sporanox for itraconazole; Mitozytrex for mitomycin) or SBE-β-CD (Abilify for aripiprazole; Vfend for voriconazole; Geodon

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for ziprazidone, and Cerenia for maropitant [veterinary use]) as solubilizing agents approved by the Food and Drug Administration (Stella and He, 2008). In practice, however, it can be found that the solubilization capacity of cyclodextrins alone may be insufficient to accommodate a therapeutic dose of the drug. This may be due to the formation of a complex with a low binding constant or due to the limited aqueous solubility of the complexing agent. In this instance, a combination of approaches may be useful: for example, pH adjustment and/or co-solvency (Chang and Shojaei, 2004).

Ricobendazole (RBZ), also known as albendazole sulphoxide, is a benzimidazole anthelmintic used in veterinary practice. RBZ is currently available as 10 or 15% (w/v) solutions for injection (Lanusse et al., 1998; Cristofol et al., 2000, 2001; Formentini et al., 2001). The injections are formulated as highly acidic solutions, since RBZ is a weak base with a pK_a of 3.5 (Wu et al., 2005a) with additional solubilization by organic co-solvents. The absolute bioavailability of these products after subcutaneous injection in cattle has been reported to be ~40% (Cristofol et al., 2001; Formentini et al., 2001). This low bioavailability was attributed to post-injection drug precipitation followed by a slow and incomplete re-dissolution at the injection site. One study reported appearance of drug precipitation at the injection site and suggested this to be the major cause of local irritation, rather than the low pH of the formulation (Formentini et al., 2001). Our preformulation work (Wu et al., 2005a) showed that RBZ is poorly soluble in water (62 μg/ml) and oils, and only slightly soluble in most of the injectable co-solvents or surfactants. Binding between RBZ and HP-β-CD is weak (binding constant 311 M^{-1}) and a solubility of 1.56 mg/ml achieved in 0.1 M HP-β-CD was considered too low to prepare a solution formulation, given the dose of RBZ is typically >50 mg/kg. As an amphoteric compound with a basic pK_a 3.5 and an acidic pK_a of 9.8, sufficient solubility for an injectable formulation (>50 mg/ml) could only be achieved at a pH lower than 1.3. At this pH the chemical stability of RBZ was found to be equivalent to that at neutral pH (Wu et al., 2009). *In vitro* studies (Wu et al., 2005b) suggested that the low pH solution would readily precipitate upon dilution with buffer pH 7.4, but that the addition of HP-β-CD into the formulation would produce a significant concentration-dependent inhibitory effect on precipitation. Therefore, a 5% RBZ injectable (solubilized by hydrochloric acid, pH ~1.5) containing 20% (0.15 M) HP-β-CD was developed with the aim of minimizing irritation and post-injection drug precipitation. A preliminary animal study (Wu et al., 2010) suggested that the bioavailability of RBZ was improved using this formulation. If the drug bioavailability could be improved, a smaller dosage of RBZ may be equally effective and a formulation at lower concentration in turn would reduce the potential of irritation.

The objectives of this study were to assess the pharmacokinetics and tissue compatibility of the HP-β-CD formulation at low pH after subcutaneous administration in sheep. Low pH solublized RBZ without CD was used as reference. The tissue tolerance to the vehicles was also assessed by observation of signs of inflammation and histological examination of the injection sites. Since pain reactions on injection were observed in some animals in the pilot study (Wu et al., 2010), 0.9% benzyl alcohol was added to the formulations and vehicle (reference) in the main study as a local anaesthetic (Gatlin and Gatlin, 1999). At this concentration benzyl alcohol is reported to cause no tissue damage in animals (Svendsen and Carstensen, 1996).

2. Materials and methods

2.1. Chemicals

Ricobendazole (>99.3%) was a gift from Transchem Limited, Ambernath, India.

Hydroxypropyl-β-cyclodextrin (Kleptose® HPB) was a gift from Roquette Lestrem, France. Water for injection and benzyl alcohol (BP grade) used in the formulations were provided by Bomac Laboratories Ltd. (Auckland, New Zealand). Albendazole sulfone (99.4%) was kindly donated by Uquifa Mexico, SA DE CV. All other chemicals and solvents were reagent grade (BDH Chemicals Ltd., England).

2.2. Animals

Animal studies were approved by the Animal Ethics Committee, University of Otago, New Zealand.

Female adult sheep ($n = 12$) weighing 63.7 ± 2.2 kg with no signs of disease or pre-existing visible lesions on the injection sites (back) were randomly divided into two treatment groups, a HP-β-CD formulation group ($n = 6$) and a reference group ($n = 6$). However, one sheep was withdrawn from the study due to bleeding at night-time after intravenous cannulation. Animals were housed in the University animal testing facility a week prior to and during the study with free access to water and hay.

2.3. Surgical preparation

On the day before injection of the formulations and vehicles, a 14-gauge 5.1 cm intravenous catheter was implanted in the jugular vein for blood sampling following an intravenous injection of Acezine® Ethical Agents Ltd., Auckland (acepromazine, 0.2 mg/kg) and Ketalar® Pfizer Ltd., Auckland (Ketamine, 2 mg/kg). Injection sites, 10 cm × 20 cm, on each side of the animal's back were shaved with an electric razor. The two contra lateral sites were located at the middle of the animal body from head to tail, and were at least 10 cm apart over the back.

2.4. Formulation administration and collection of blood samples

Formulations and vehicles were prepared using aseptic technique a day prior to the animal study as described previously (Wu et al., 2010) with addition of 0.9% (v/v) benzyl alcohol as a local anaesthetic to reduce the pain on injection. Briefly, RBZ was dissolved in an HCl solution at a molar ratio of 3:4. Benzyl alcohol, and HP-β-CD (for the CD formulation) were then added before making up to volume with water for injection. The vehicles were solutions containing 0.2 M HCl or 20% HP-β-CD with addition of benzyl alcohol.

After recovery for 24 h, each animal received a subcutaneous injection of the randomly assigned formulation to one side of the back at a dose of 5 mg/kg (0.1 ml/kg) and the respective vehicle on the contralateral side at a dose of 0.1 ml/kg. Formulations and vehicles were administered subcutaneously by a veterinarian using 19-gauge needles. Blood samples were withdrawn immediately before injections and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30, 36, 42 and 48 h post-injection for assay of RBZ and its metabolite (albendazole sulphone), and for creatine kinase (CK) analysis. The first 3 ml of blood was discarded before a blood sample (5 ml) was collected into a heparinized tube. Catheters were then flushed with 8 ml of heparinized saline to maintain patency. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored in Eppendorf tubes at −20 °C until analyzed.

2.5. Evaluation of injection site reactions

2.5.1. Visual observations and palpation

After subcutaneous administration of the formulations and vehicles, visual observations of the injection site were made to assess tissue reactions. In particular, attention was paid to signs of pain on injection, appearance of swelling and reactions to palpation at the injection sites over the next 48 h and daily during the week.

Table 1
Injection site reactions following subcutaneous injection of RBZ formulations and vehicles in sheep. Data are the numbers of animals in which a site reaction was observed.

Site reaction	HP-β-CD ($n=6$)		Reference ($n=5$)	
	Formulation	Vehicle	Formulation	Vehicle
Pain on injection[a]	4	0	2	1
Swelling (size of edema)	1 (2–3 cm)	0	2 (2–3 cm)	1 (2 cm)
Temperature elevation $AUC_{\Delta T}$ (h °C)	24 ± 11	20 ± 12	46 ± 26	39 ± 12
Histological change	3	0	2	1

[a] Pain response involved back stiffening and backward movements of the animals.

Pain response was classified as back stiffening (slight pain), backward movement (moderate pain) and jumping to indicate severe pain.

2.5.2. Skin temperature at the injection site

Skin temperature at the injection site was measured using an infrared non-contact thermometer before injection, and at times over 48 h post-injection. A circadian rhythm in the skin temperature was observed in the sheep. Therefore, throughout this study for each sheep a reference site at least 10 cm away from the injection site, was taken in order to account for the fluctuations in normal skin temperature. Skin temperature elevation (ΔT) at the injection site compared to the reference site and the areas under the ΔT–time curves ($AUC_{\Delta T}$) were compared for each treatment.

2.5.3. Plasma creatine kinase concentration

Concentration of CK in plasma before drug administration and at times, 2, 4, 6, 9, 12, 18 and 24 h were analyzed within 48 h after sampling by Gribbles Veterinary (Dunedin, New Zealand) using a commercial ELISA assay kit (Roche Diagnositics, Auckland, New Zealand).

2.5.4. Tissue histology of excised injection site

On day seven after drug administration, following an intravenous injection of Acezine® and Ketalar®, tissues from the injection sites including epidermis, dermis and hypodermis were taken by punch biopsies and examined by a histologist who was blinded to the treatments.

2.6. Pharmacokinetic study

2.6.1. Analytical procedures for RBZ and albendazole sulfone

A validated reversed phase HPLC method was used for quantification of RBZ and its main metabolite albendazole sulfone ($ABZSO_2$) in the plasma. The HPLC assay and method for sample preparation with solid phase extraction were as described previously (Wu et al., 2005c).

2.6.2. Pharmacokinetic calculations

Pharmacokinetic parameters were estimated using non-compartmental analysis based on statistical moment theory (Gabrielsson and Weiner, 2000). The maximum concentration C_{max} and the time when it occurred, t_{max}, were observed directly. Terminal half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/\lambda$, where λ is the slope obtained from the regression of natural log concentration versus time in the terminal phase over the last four data points. The linear trapezoidal rule was used to calculate area under the curve (AUC) and the area under the first moment curve (AUMC). AUC and AUMC extrapolated beyond the last drug concentration data point (C_n) to infinite time were obtained from C_n/λ and C_n/λ ($t_n + 1/\lambda$), respectively, and were used to calculate the mean residence time (MRT). Relative bioavailability (F) of the HP-β-CD formulation compared to the reference formulation was estimated by comparison of $AUC_{0-\infty}$.

2.7. Statistical analysis

Pharmacokinetic data analysis was performed using GraphPad Prism for Windows, version 4.01 (GraphPad, San Diego, CA, USA). Skin temperature and pharmacokinetic parameters of the two groups were compared by ANOVA using Minitab for Windows, version 12.1 (Minitab Inc., PA, USA). The P value for significance was set at 0.05.

3. Results

3.1. Evaluation of injection site reactions

3.1.1. Visual observations and palpation

Slight or moderate pain reactions were observed in some sheep following injection of the reference vehicle, and both experimental formulations: no pain reaction was seen with injections of the HP-β-CD vehicle alone. Apart from transient swelling of the skin observed shortly after the injections in some sheep, which disappeared in 2–5 h, no injection site reactions were seen (Table 1).

3.1.2. Plasma creatine kinase concentration

The overall activity of plasma CK remained in the range of pre-injection level for all the animals over the 48 h post-injection (Fig. 1). CK concentrations were slightly higher in the HP-β-CD group than in the reference formulation group but not significantly different compared to the pre-treatment concentrations ($P > 0.05$).

3.1.3. Skin temperature at the injection site

Skin temperatures at the injection sites and reference site over 48 h post-injection were in the range 33–36 °C. A transient hypothermia at 0.5 h after injection was observed at the injection

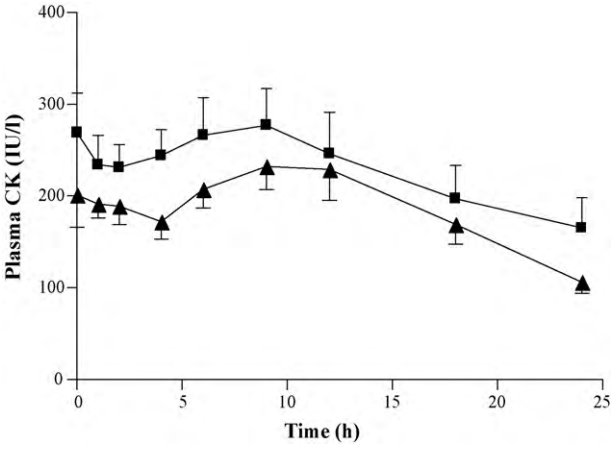

Fig. 1. Plasma creatine kinase (CK) after s.c. administration of the HP-β-CD formulation (squares, $n=6$) and the reference formulation (triangles, $n=5$), along with the respective vehicles at a dose of 0.1 ml/kg. Data are means ± standard error.

Table 2
Comparison of non-compartmental pharmacokinetic parameters (means ± SD) of RBZ following s.c. administration of the HP-β-CD formulation (CD) ($n = 6$) and the reference (R) ($n = 5$) in sheep at a dose of 5 mg/kg RBZ base.

Parameters	HP-β-CD	Reference	Group ratio (CD/R)	*P* value
C_{max} (μg/ml)	2.90 ± 0.77	1.34 ± 0.31	2.2	<0.05
t_{max} (h)	5.0 ± 0.6	9.6 ± 2.9	0.5	<0.01
k (h^{-1})	0.15 ± 0.06	0.09 ± 0.04	1.6	0.051
$t_{1/2}$ (h)	5.5 ± 2.8	8.5 ± 3.4	0.6	>0.05
$AUC_{0-48\,h}$ (μg h/ml)	53.5 ± 14.0	35.0 ± 8.3	1.5	<0.05
$AUC_{0-\infty}$ (μg h/ml)	54.5 ± 15.3	36.7 ± 9.2	1.5	<0.05
MRT (h)	14.7 ± 1.3	17.1 ± 1.8	0.8	<0.05

sites in most of the animals, followed by a temperature increase. A second hypothermia was observed in the formulation sites (Fig. 2). The HCl vehicle caused a constant hyperthermia after administration, with a maximum elevation of 1.7 °C, returning to normal after approximately 48 h. The areas under the ΔT–time curves ($AUC_{\Delta T}$) are shown in Table 1. Repeated measures analysis of variance of $AUC_{\Delta T}$ showed a significant difference between the two RBZ formulations ($P < 0.05$) whereas the presence or absence of RBZ had no effect on the $AUC_{\Delta T}$ ($P > 0.05$).

3.1.4. Tissue histology of excised injection site

No morphological change was observed in the biopsy samples from the injection sites treated with the 20% HP-β-CD solution. Whereas minimal or mild perivascular dermatitis, steatitis and inflammatory cell infiltration was seen in most of the tissue samples treated with the reference formulation or vehicle, and in one of the six samples treated with the HP-β-CD formulation. Some tissue samples showed minimal to mild necrosis in the subcutis or muscle (Fig. 3; Table 1).

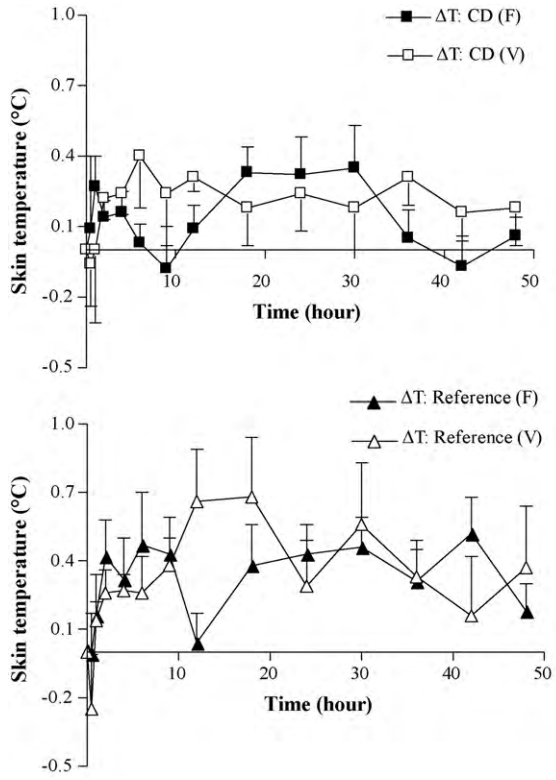

Fig. 2. Relative skin temperatures change at the injection sites compared to the reference sites (ΔT) following subcutaneous injection of two RBZ formulations (F) and vehicles (V) in sheep. Data are mean values with standard error bars.

3.2. Pharmacokinetics of RBZ

Following subcutaneous injection, RBZ was detectable in the first blood samples taken at 15 min in the HP-β-CD group. However, in the reference group it was only detected after 30 min or 1 h followed by a plateau after the t_{max} (9.6 ± 2.9 h) in the drug concentration–time profile. The HP-β-CD group showed higher RBZ plasma concentration than in the reference formulation group (Fig. 4, Table 2): AUC and C_{max} of the HP-β-CD formulation were 1.6 and 2.2 times higher than the reference formulation, respectively, whereas t_{max} was significantly shorter, suggesting HP-β-CD significantly increased the rate and extent of absorption of RBZ ($P < 0.05$). The overall concentrations of the main metabolite $ABZSO_2$, detectable at 0.5 and 1 h for the HP-β-CD formulation and the reference formulation, respectively, were proportionally lower than the RBZ concentrations.

4. Discussion

The effectiveness of CD in enhancing the bioavailability of oral formulations has been addressed (Szejtli, 2005); however, little is known about the biopharmaceutics of injectable dosage forms. In the present study, the comparative tissue compatibility and bioavailability of the poorly soluble drug, RBZ, solubilized either by low pH alone or by low pH with HP-β-CD, were simultaneously investigated and the role HP-β-CD of has been highlighted.

The pharmacokinetic studies revealed a plateau in the drug concentration–time profile in the low pH solubilized formulation with a delayed t_{max} (~10 h), which is similar to that reported by Formentini et al. (2001) for the commercial product Sintyotal-R® after s.c. injection in cattle. Compared to those of the reference formulation, AUC and C_{max} of the HP-β-CD formulation were 1.6 and 2.2 times higher, respectively. AUC of the HP-β-CD group is nearly identical to the data reported by Goudah (2003) after intravenous administration of RBZ at the same dose in sheep, suggesting RBZ absorption from the HP-β-CD formulation may be virtually complete.

There are two possible mechanisms by which HP-β-CD enhanced RBZ absorption. Firstly, as suggested in an *in vitro* study (Wu et al., 2005b), HP-β-CD may inhibit drug precipitation at the injection site by stabilizing the super-saturated solution created at the injection site when the injection is diluted and neutralized by the tissue fluids. HP-β-CD has been reported to stabilize the super-saturated solutions of a number of poorly water soluble drugs (Torres-Labandeira et al., 1991; Iervolino et al., 2001; Dias et al., 2003; Brewster et al., 2008), and a high drug concentration would favour drug absorption by passive diffusion. Secondly, HP-β-CD has been proposed as a potent absorption enhancer of drugs (Albers and Müller, 1995) by an as yet poorly understood mechanism (Masson et al., 1999). In general, it is believed that cyclodextrins enhance drug permeability by solubilizing their lipophilic components, thereby disrupting barriers to diffusion and increasing permeability. Other studies (Masson et al., 1999; Sridevi and Diwan,

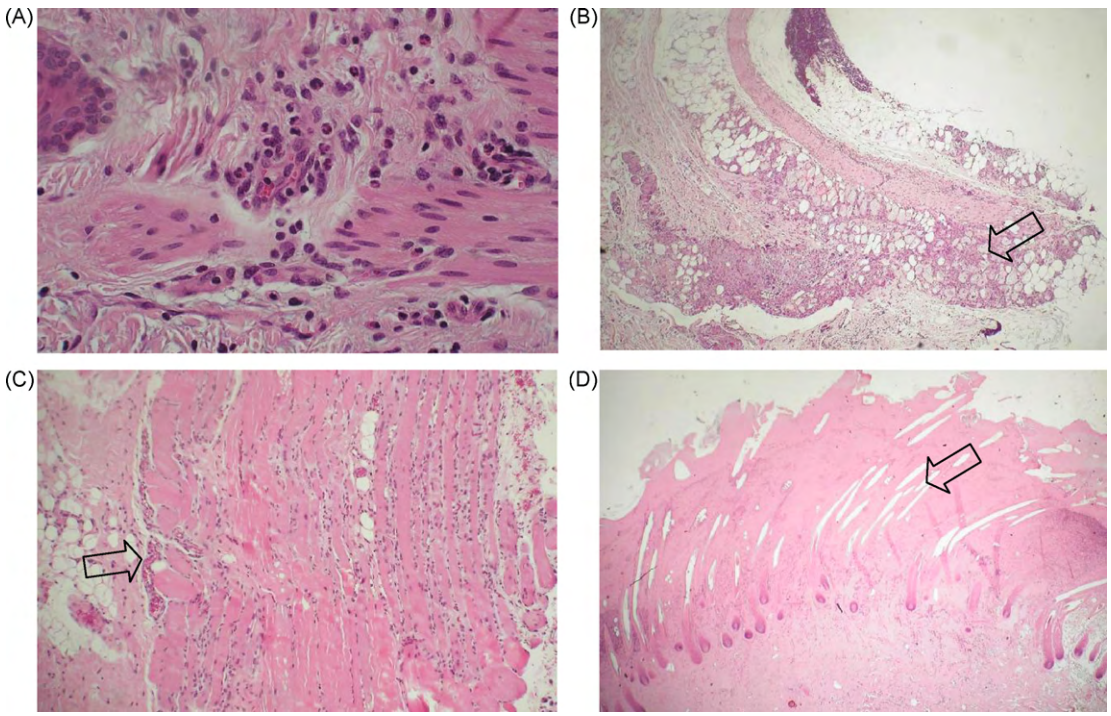

Fig. 3. Microscopic observation of tissue damage at the injection sites indicated by arrows. (A) Perivascular dermatitis showing inflammatory cell infiltration; (B) steatitis; (C) muscle damage showing inflammatory cell infiltration and necrosis; and (D) dermal necrosis.

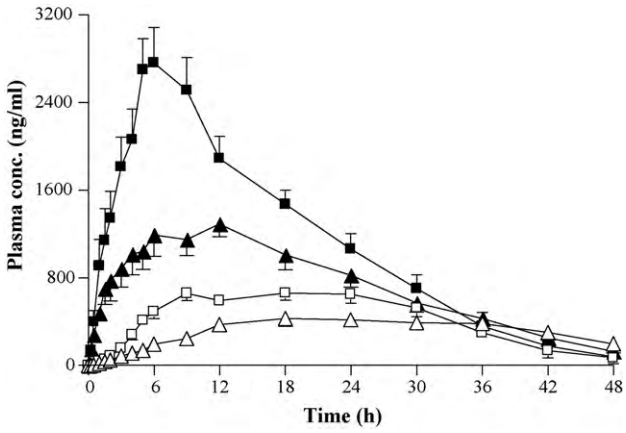

Fig. 4. Mean plasma concentration–time profiles of RBZ (solid symbols) and $ABZSO_2$ (open symbols) after s.c. administration in sheep of the reference formulation (triangles, $n = 5$) and the HP-β-CD formulation (squares, $n = 6$) at a dose of 5 mg/kg RBZ base. Data are means ± SE.

2002) suggested that cyclodextrins act as permeation enhancers by carrying the drug in the inclusion complexes through the aqueous barrier, from the bulk solution towards the surface of biological membranes.

The disappearance of plasma RBZ appears to follow first-order kinetics, but the slopes of the terminal phase for the two formulations were different. The HP-β-CD formulation gave a shorter apparent half-life (5.5 ± 2.8 h) than the reference formulation (8.5 ± 3.4 h), but fairly close to that for i.v. administration (5.0 ± 0.75 h) reported by Goudah (2003). This difference could be due to increased elimination rate of the drug from the blood in the case of the HP-β-CD formulation, leading to the shorter half-life, or to a slow release of precipitated RBZ from the reference formulation leading to the longer half-life. Since a significant alteration in elimination parameters only occurs if the drug has extremely high affinity (binding constant $>10^5\ M^{-1}$) with the CD in the blood (Perry et al., 2006; Stella and He, 2008), which is not the case with RBZ, the former would seem unlikely; therefore, it is most likely that the difference in the terminal profiles is explained by the relatively slow and incomplete absorption of the reference formulation as a result of post-injection drug precipitation. Similarly, the longer MRT observed for the reference formulation ($P < 0.05$) is likely to be the result of slow re-dissolution of precipitated RBZ.

Both formulations resulted in pain on injection and swelling in some of the animals. Tissue histology results suggest that minimal to mild necrosis occurred in some of the animals in both formulations. It should be noted however that biopsies, taken 1 week after drug administration reflect subacute inflammatory responses rather than chronic reactions caused by drug precipitation. Several studies (Manor and Sadeh, 1989; Mikaelian et al., 1996; Sutton et al., 1996) have suggested that morphological changes do not increase in severity beyond 1 week after injection, and that the minor changes seen in the present study could be expected fully to resolve within 2–3 weeks. Indeed, lack of elevation of plasma CK, even though the respective vehicle was injected simultaneously, indicates that the RBZ injectables caused no significant damage to the underlying muscle tissue in sheep. Plasma CK has been found to be reliable a parameter to monitor the extent of muscle damage after intramuscular injections in species such as dog, sheep, horse and cattle (Wilkinson, 1970; Toutain et al., 1995; Lefebvre et al., 1996). The method has also been used to estimate the damage to superficial muscle caused by s.c. administration in calves (Kaartinen et al., 2000), where the maximal CK levels at 8–12 h post-injection were found to be >10 times higher than the pre-injection levels. Addition of benzyl alcohol appeared to reduce the pain on injection of the reference formulation and vehicle. However, the local anaesthetic effect of benzyl alcohol was not obvious in the presence of HP-β-CD. This may be because benzyl alcohol complexed with HP-β-CD reducing the free concentration for a local anaesthetic effect: it has been shown previously that benzyl alcohol complexes with β-CD in an aqueous solution (Varady et al., 2002).

Subcutaneous injection of 20% HP-β-CD was shown to be well tolerated by sheep, suggesting HP-β-CD is a good formulation carrier for local injection in this species. Generally, tissue irritancy by cyclodextrins is attributed to their solubilizing effect on biological membrane components, especially cholesterol. This effect of cycloedextrins is minimized in HP-β-CD as it has a low affinity for lipophilic membranes (Rajewski and Stella, 1996). The protection effect of HP-β-CD against cytotoxicity of RBZ observed *in vitro* (Wu et al., 2010) was not observed in sheep possibly because most of the RBZ in the formulation (94.6%) is not complexed. Furthermore, addition of HP-β-CD to the formulation caused an increase in tonicity (Zannou et al., 2001), which may have contributed to pain on injection, yet resulted in minimal morphological change, as has previously been reported by Formentini et al. (2001) for an acidic solution. Acid solutions are thought to stimulate nociceptors and initiate neurogenic inflammation (Bevan and Geppetti, 1994), and are less likely than high pH solutions to cause site reactions (Simamora et al., 1995), although the mechanism is still unclear.

In this study, skin temperature change was used as one of the clinical signs of inflammation at the injection site. It has been used for early detection of phlebitis induced by intravenous injection into rabbit ears (Ward et al., 1991). Similar to the previous study (Wu et al., 2010), a circadian rhythm in the skin temperature was observed. In addition, environmental temperature has a direct influence on the animal skin temperate (Lee et al., 1941). To account for the fluctuations in normal skin temperature, a reference skin site was taken and used to estimate the skin temperature variation at the injection site. The transient hypothermia, which occurred at 0.5 h, may be due to a transient vasodilation followed by a net decrease in blood flow in precapillaries in response to the injected materials (Chandrasoma and Taylor, 1991). The delayed hypothermia might be the result of a transient blockage of blood vessels due to drug deposition, or a transient loss of temperature regulation due to tissue injury.

5. Conclusions

In conclusion, the HP-β-CD formulation showed promise for subcutaneous injection for RBZ to increase bioavailability and potentially minimize injection site reactions. HP-β-CD is a useful tissue-compatible excipient for poorly water soluble drugs in injectables. A bioavailability enhancement of 1.6 times would allow a reduction in the dose of HP-β-CD formulation to achieve the same efficacy, thus attenuation of local irritation could be anticipated by either injecting a smaller volume of the formulation or reducing the concentration of RBZ in the injection. Then tissue damage induced by drug precipitation can also be circumvented.

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