Investigation into the Topical Delivery of Diclofenac into Equine Skin

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ABSTRACT SUMMARY

The aim was to evaluate a novel diclofenac formulation designed to control mild-to-moderate inflammation and pain associated with orthopedic and soft tissue conditions in horses. Delivery from the new formulation was compared to that from a reference product (Surpass®) using skin from five healthy horses. Cumulative permeation and skin sequestration were significantly increased with the new formulation. Mathematical modeling showed that this was due to a significant increase in drug partitioning into the skin.

INTRODUCTION

Osteoarthritis (OA) is a common and debilitating disease state in racehorses. It was reported to be the most prevalent complaint among long-term/recurrent conditions evaluated in a survey in Great Britain (14%) and one of the most common causes of lameness in the U.S. (up to 60%). OA can be caused by trauma, simple “wear-and-tear”, or faulty biomechanics that place undue stress on a given joint and can become a chronic condition. Given the presence of inflammation, it follows that non-steroidal anti-inflammatory drugs (NSAIDs) are routinely used to treat joint disease in the horse [1]. Diclofenac is a potent, non-selective cyclo-oxygenase inhibitor and is the most widely used NSAID in humans and there have been several studies in vitro and in vivo evaluating its potential application to treat OA in horses. However, the results have been mixed. For example, previous investigations using a formulation containing 1.16% diclofenac diethylamine, reported a lack of pharmacological efficacy in horses with OA [2]. Formulations designed for delivery to human skin might not perform as well with equine skin.

Surpass® cream contains 1% diclofenac sodium and has been shown to be effective in controlling pain and inflammation in horses suffering from OA. However, the amount of cream that must be applied and the need to rub it into an area that is inflamed and painful, mean that there is an opportunity to develop more potent and easier to apply formulations.

The objectives of this study were (i) to investigate the topical delivery of diclofenac from a proprietary 1.3% diclofenac epolamine formulation, (ii) to compare results with those using Surpass® and (iii) to provide a mechanistic interpretation of differences between the behavior of the two formulations by fitting the data to a solution of Fick’s Second Law of diffusion and hence to determine the partition and diffusion parameters [3].

EXPERIMENTAL METHODS

Equine skin from 5 different donors (aged 1 to 8 years) was obtained from a local abattoir and stored at -20°C until use which was within 1 week of harvesting. Prior to use, hair was removed with electric clippers – but not shaved – and sliced to a uniform thickness (2.2 ± 0.2 mm). Experiments were performed using conventional Franz diffusion cells (area 2 cm²).

Three different formulations were evaluated: the novel 1.3% liquid diclofenac epolamine formulation (Wezen Bio AG; WB), a marketed formulation (Surpass® cream; Boehringer Ingelheim Vetmedica, Inc.) and a 1% aqueous solution of diclofenac sodium. Formulations were applied to the external surface of the equine skin samples (200 mg per cm² for WB and Surpass® and 200 μl per cm² for the drug in aqueous solution; 1.87 mg of diclofenac per cm²) and left in place for 24 h. The receiver compartment was filled with phosphate buffered saline (PBS; pH 7.4). Aliquots of 1 ml were withdrawn at specific time-points. Each aliquot was replaced with fresh buffer. After completion of the experiment, the formulation was removed from the donor compartment and the skin surface cleaned with running water and with a cotton swab to remove any residue. Then, diclofenac retained in the skin was extracted using a previously validated skin extraction procedure. Each formulation was evaluated using skin from 5 different donors with 6 replicates per donor.

Diclofenac was assayed by HPLC-UV using a P680A LPG-4 pump equipped with an ASI-100 autosampler, a UVD 170U UV/Vis detector (Dionex, Voisins Le Bretonneux, France) and a Lichrospher® column (125 x 4 mm) packed with 5 μm C18 silica reversed-phase particles. The mobile phase comprised 0.05 M KH₂PO₄ (pH 5.5): acetonitrile (45:55 v/v). The flow rate was 1.0 ml/min, the column temperature was 40°C and the injection volume was 75 μl. The detection wavelength was 205 nm.

Data were expressed as the mean ± SD. Outliers determined using the Grubbs test were discarded. Results were evaluated statistically using ANOVA or Student’s t-test.

RESULTS AND DISCUSSION

The equine skin used in these studies was from five healthy horses, with an age range of 1 to 8 years. In order to evaluate whether the age of the animal affected diclofenac delivery, two groups were created: 1 year (2 donors) and 6 to 8 years (3 donors). Although the age of the donor did not seem to be a relevant factor when
diclofenac was administered from either aqueous solution or when using Surpass®, there was a clear difference for the WB formulation (Figure 1).

In order to explain the differences between the amounts of drug delivered, the partition and diffusion parameters were estimated by fitting the cumulative permeation data to the appropriate solution of Fick’s Second Law:

$$Q = (KH) \cdot C_{veh} \left[ \frac{D}{H^2} \left(1 - \frac{1}{6} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^3} \exp \left(-\frac{Dn^2 \pi^2 t}{H^2} \right) \right) \right]$$

where $Q$ is the cumulative amount of drug permeated per unit area at time $t$, $C_{veh}$ the concentration of drug in the donor vehicle, $K$ the stratum corneum/vehicle partition coefficient, $D$ the diffusion coefficient, and $H$ the diffusion pathlength. Although there was no dependence on the diffusion parameter ($D/H^2$), a linear correlation was observed between the diclofenac steady state flux and the partition parameter ($KH$) (Figure 2).

Figure 1. Steady state flux of diclofenac as a function of age of the horses. Data presented as mean ± SD; n≥11.

Statistically significant differences compared to “Solution” (ANOVA; p<0.001) **Statistically significant differences compared to “Surpass” (ANOVA; p<0.001) ***Statistically significant differences compared to “Wezen Bio formulation 6-8 years” (ANOVA; p<0.001).

Figure 2. Correlation between steady state transdermal flux of diclofenac and the partition parameter ($KH$).

Figure 3. Skin deposition of diclofenac as a function of age of the horses. Data presented as mean ± SD; n≥11.

**Statistically significant differences compared to “Solution” (ANOVA; p<0.05) ***Statistically significant differences compared to “Surpass” (ANOVA; p<0.001).

However, in this case, the age of the horses was not a relevant factor.

The transdermal flux of a molecule depends on its thermodynamic activity (degree of saturation) in the formulation. The WB formulation contains volatile components that evaporate upon application to the skin, thereby increasing the drug concentration and hence thermodynamic activity in situ. This favors partitioning from the formulation into the skin and so increases drug flux. In a next step, we will investigate the application of this liquid formulation as a topical spray. The aim is to achieve controlled therapeutic delivery of diclofenac to the soft tissue and articulations while ensuring that systemic exposure is reduced compared to Surpass®.

CONCLUSION

Quantification of diclofenac delivery (permeation and deposition) into equine skin demonstrated the superiority of the new formulation over the reference product, Surpass® cream. Mechanistic analysis revealed that this was due to increased partitioning of diclofenac into the skin. These preliminary results also suggested that the age of the animal influenced diclofenac permeation from the WB formulation but was not a factor in its deposition. In vivo studies have recently been initiated to test pharmacological efficacy.

REFERENCES


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