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Ex-vivo permeation of enrofloxacin through shed skin of *Python molurus bivittatus*, as evaluated with a Franz cell

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ABSTRACT

Current treatment options for snake infections are straightforward for experienced veterinarians, but are less so for non-veterinarian snake owners. In this study, we analysed the *in-vitro* permeation of an antibiotic, enrofloxacin, administered percutaneously to the shed skin of a snake (*Python molurus bivittatus*) using a Franz cell. The test formulation was based on the incorporation of enrofloxacin (5 wt %) into a commercial vehicle, Pentravan® cream. This cream is a hydrophilic emulsion that uses liposomal technology to promote transdermal drug delivery. Two different parts of the shed skin were tested; the first part was close to the head and the second part was just before the cloaca. The amount of enrofloxacin absorbed through the skin was assayed by reversed-phase high-performance liquid chromatography (HPLC). Results seemed to indicate that delivery of enrofloxacin was greater across the skin sampled close to the head than across the skin sampled close to the cloaca. This is due to the lower scale: hinge ratio associated with skin from close to the head. This study confirmed that enrofloxacin can be absorbed through the shed skin of snakes and investigated the percutaneous route as a new option of administering drugs when treating snakes.

1. Introduction

From the time that herpetological medicines were developed, reptiles have been administered drugs through the same routes, including intramuscular (IM), intravenous (IV injection), or oral (PO, *per os*) routes. These administrative routes are straightforward for experienced veterinarians, but are less so for non-veterinarian owners, who may have to perform injections or oral dosing [10–12,18,19]. It is therefore important to find a simpler route for drug administration. This would facilitate effective treatment by the snake owners, especially in cases where extended periods of drug treatment are required.

The transcutaneous route offers the most non-invasive and painless option for drug treatments [1,5,17]. This study therefore investigated this route as a possibility for administration of drugs to snakes. Interestingly, reptile skin has been used in drug delivery tests as a possible model for human skin [6,9,14]. However, to our knowledge, transcutaneous delivery options have not been considered for snakes. This study is therefore the first to investigate the possibility of transcutaneous passage of a drug widely used in herpetological medicine.

Enrofloxacin (1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinoline carboxylic acid, Fig. 1) was used as the test drug. Enrofloxacin is an antibiotic belonging to the fluoroquinolone family [3,4,7,10] and is commonly administered to

Abbreviations: APIs, Active Pharmaceutical Ingredients; IM, IntraMuscular route of administration; IV, IntraVenous injection; PO, *Per os* or oral route of administration; SC, *Stratum Corneum*; TEWL, TransEpidermal Water Loss; UL, Unilamellar Liposomes.

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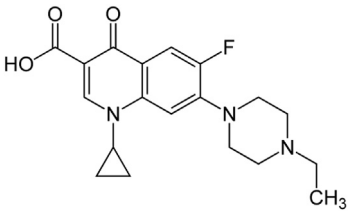

Fig. 1. Chemical structure of enrofloxacin.

snakes. For the transdermal drug vehicle, Pentravan® cream, an oil-in-water emulsion that uses liposomal technology to ensure reproducible transdermal penetration of active ingredients was used [8,13].

Prior to initiating the experiment, it was important to consider the optimal area of shed snake skin for enrofloxacin application. Previous studies [20] concluded that the back (dorsal) of shed snake skin, which is thicker than shed belly (ventral) skin, is the best option for drug-permeation studies. This formed the basis of our decision to select the back of the shed skin of *Python molurus bivittatus* in order to study the permeation of enrofloxacin. For comparison, drug permeation was also evaluated using porcine skin, which is a conventional *ex-vivo* model, often used in these types of experiment.

Overall, the goal of the study was to develop the transcutaneous route as a simple viable route for the administration of drugs, including antibiotics, such as enrofloxacin, in herpetological medicine. This would facilitate simple effective treatments especially in cases where extended periods of drug therapy are required.

2. Materials and methods

2.1. Materials

Enrofloxacin hydrochloride and Pentravan® cream were provided by Fagron (Rotterdam, Netherlands). The Pentravan® cream composition and the functional categories of its ingredients are reported in Table 1. Gradient grade Acetonitrile and hydrochloric acid were purchased from VWR® (Fontenay Sous Bois, France), perchloric acid (70%) from Sigma Aldrich (St. Louis, MO, USA), and phosphoric acid (85%) from Merck (Rahway, NJ, USA). Deionized water was obtained by purification through the Purelab Option (ELGA LabWater, High Wycombe, UK).

2.2. Methods

2.2.1. Shed snake skin samples

The shed snake skins of two specimens of *Python molurus bivittatus* were used in this study. Skins were obtained from the acclimatization centre of "La ferme tropicale" (Combs-la-ville, France). They were stored at room temperature until their use in the experimental studies. Samples were taken from the back of the shed skin. Samples were hydrated by the receptor solution for 30 min after being placed on the Franz cells.

2.2.2. Porcine skin samples

The porcine skin was dermatomed from pig ears obtained from a local abattoir (Lyon, France) within 12 h after sacrifice. The pig ears were washed with demineralized water and gently wiped with paper tissues, then stored in sealed plastic bags in a freezer set to maintain −20 °C. Before their use in the experimental studies, the pig ears were thawed at ambient temperature for 2 h.

2.2.3. HPLC quantification of enrofloxacin

The concentrations of enrofloxacin in receptor solution samples were determined by means of an HPLC assay. Each receptor solution sample (0.5 mL) was added to an Eppendorf tube containing 1 mL of acetonitrile. The sample was then mixed and allowed to stand for a further 30 min before being centrifuged (8400 rpm, 6000 × *g*) for 20 min. Supernatant (600 μL) was transferred to a vial. Perchloric acid, 70% (30 μL) was added prior to sample analysis. From an enrofloxacin stock solution, dilutions were made in assay buffer, to prepare a calibration curve ranging from 3×10^{-6} M to 600×10^{-6} M (Fig. 2).

The calibration and receptor solution samples were analysed as follows: the HPLC system was a Water Alliance 2795 instrument with a UV DAD Waters 2996 detector (set at 280 nm). Chromatographic separation was performed on a Kinetex™ 5 mm C_{18} 100 Å column (150 mm length × 4.6 mm internal diameter). The mobile phase was a mixture of acetonitrile/phosphoric acid 0.002 M (83:17, v/v) run in isocratic mode at a flow rate of 0.5 mL min^{-1}. The mobile phase was filtered through a 0.45 μm membrane filter (Whatman®, diameter = 13 mm). The total run time was 13 min. The injected sample volume was 15 μL. The HPLC system was operated at ambient temperature (25 ± 1 °C).

2.2.4. Enrofloxacin formulation

Enrofloxacin was directly incorporated at 5 wt % in Pentravan® cream vehicle according to the good manufacturing practices of veterinary compounding. Pentravan® is an oil-in-water vanishing cream that uses liposomal technology to ensure its reproducibility

Table 1
Pentravan® cream ingredients and their functional category.

Ingredients	Functional category
Simethicone	Antifoaming agent
Benzoic acid	Antimicrobial preservative
Potassium sorbate	Antimicrobial preservative
Sorbic acid	Antimicrobial preservative
Butylated hydroxytoluene	Antioxidant
Hydrochloric acid	Buffer
Soya lecithin	Emollient; emulsifying agent; solubilizing agent
Isopropyl myristate	Emollient; oleaginous vehicle; skin penetrant; solvent
Polyoxyl 40 stearate	Emulsifying agent; solubilizing agent; wetting agent
Cetyl alcohol	Emulsifying agent; stiffening agent
Carbomer 980	Gelling, stabilizing agent
Pure water	Solvent
Stearyl alcohol	Stiffening agent
Glycerol	Wetting agent