In Vitro Studies on Aminoglycosides Permeation and Resorption in Porcine Skin as a Model Membrane

W. A. Awad*‡ and J. Zentek‡

*Clinic for Avian, Reptile and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, A-1210 Vienna, Austria, ‡Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, 14195 Berlin, Germany

ABSTRACT

The in vitro study of drug skin permeability plays an essential role in the selection of drugs for the development of transdermal dosage forms. Therefore, the objective of present study was to state the dermal penetration, permeation and absorption of topically applied substances (Gentamicin and Polymyxin B sulphate). Skin samples of the ear and umbilical region of porcine were used in the experiments. At the beginning of the experiment, 500 µL of drug gel formulation was placed on the external side of the skin. After elapsed times of 30, 60, 90 and 120 min, 1.0 mL of the receiving solution was withdrawn and replaced with an equal volume of fresh buffer. The concentrations of each compound in the receiver medium were determined by high-performance liquid chromatography (HPLC). The results of the present study showed that all samples were under the detection limit. In conclusion, the systemic absorption for gentamicin and polymyxin B sulphate is poor when used topically.

Key words: antibiotics; gentamicin; polymyxin B sulphate; drug resorption; ototoxicity; porcine skin

INTRODUCTION

The in vitro study of drug skin permeability plays an essential role in the selection of drugs for the development of transdermal dosage forms. In the setting of animal models for transdermal permeation studies, the characteristics of excised skin from porcine were thoroughly investigated and widely used. Indeed, the main barrier to drug permeation through skin is the stratum corneum, which has been reported to differ in terms of lipid composition, water content and morphological characteristics (thickness, number of pores, and follicles). In Figure 1, penetration, permeation and resorption which designate the penetration of a substance in and through the skin layer are graphically represented. Porcine stratum corneum is the most similar to human stratum corneum in terms of lipid composition. The most of drugs pass through the skin predominantly by passive diffusion (Potts et al., 1992). The topical application of aminoglycoside antibiotic preparations containing gentamicin (Figure 2a) and polymyxin B sulphate (Figure 2b) is used normally in the treatment of chronic or acute Otitis media and Otitis externa. Furthermore, systemically absorbed aminoglycosides induce ototoxicity in human beings and in animals (Barlow et al., 1994). It has been found that systemic absorption of topical gentamicin can be occurred producing a serum gentamicin level of 6.2 µg/mL (Green et al., 1997).

Gentamicin is a positively charged aminoglycoside antibiotic. In hair cells,
aminoglycosides have long half-lives (5–6 months) and are poorly degraded (Dulon et al., 1993; Imamura and Adams, 2003). However, serious nephrotoxic and ototoxic side effects can occur when aminoglycosides are used clinically (Barza and Lauermann, 1978; Lerner and Matz, 1979).

The objective of the present study, therefore, to represent the topical application of gentamicin and polymyxin B sulphate in a comparing investigation of the transdermal resorption and to assess the relevance of animal data to the use of ototopical drops in clinical situations involving humans.

MATERIALS AND METHODS

Skin Preparation

The in vitro skin permeation studies were performed in 6 replicate mounted on Ussing chamber with an effective diffusion area of 3 cm². The experiments were performed using the porcine skin. The subcutaneous fat was carefully removed, and the skin was cut into 3 cm² samples. The skin samples were taken from umbilical abdominal region and from the internal skin of the ear. The skin was incubated with mannitol buffer solution with the following composition (mmol/L): CaCl₂, 1.5; MgCl₂, 1.2; Na₂HPO₄, 2.4; NaH₂PO₄, 0.6; NaHCO₃, 25; KCl, 5; NaCl, 115; mannitol, 20. The pH of the solution was adjusted to 7.4 using a pH meter. A circular specimen of the skin was sandwiched securely between the 2 halves of the chamber, with the stratum corneum side facing the external side. The internal side was filled with the buffer solution (40 mL), thermostated at 37°C, and continuously stirred by gassing with carbogen.

At the beginning of the experiment, 500 µL of drug-solution [gentamicin, (13200 IU/mL) and polymyxin B sulphate, (3.48 mg/mL)] was placed on the external side of the skin samples mounted on the Using chambers. After elapsed times of 30, 60, 90 and 120 min, 1.0 mL of the receiving solution was withdrawn and replaced with an equal volume of fresh buffer. The concentrations of each compound in the receiver medium were determined by high-performance liquid chromatography (HPLC).

Analysis of Aminoglycosides

For the concentration and cleanup of the samples and the gentamicin sulphate standards solid phase extraction (SPE) with cation exchange was used. The analysis of the samples and the gentamicin standard was performed by HPLC-LC/MS. The gentamicin sulphate standard was prepared by dissolving it in mannitol buffer with a final concentration of 5 µg/ml and 20 µg/ml, respectively. Sample or standard solution were washed two times (with water and Methanol/Acetonitrile (50/50) and centrifuged in a 50 mL Greiner-tube for 15 min with 4,350 rpm. The samples were evaporated in a vacuum concentrator at 45°C and 600 mbar and reconstituted in 200 µl of 95/5 Acetonitrile/100 mM Ammonium formate, pH 3.2. Twenty microliters of reconstituted aliquot was injected into the HPLC system with a flow rate of 0.5 mL/min. Separation of analytes was performed with a S113 Ultracarb RP 18 column. The chromatograms were integrated with the help of the software Agilent (6210 ESI-TOF, Agilent Technologies, Santa Clara, CA). The chromatogram of standards showing their peaks and retention times is shown in Figure 3.

RESULTS AND DISCUSSION

Ototoxicity refers to medication-caused auditory and/or vestibular system dysfunction causing hearing loss or dysequilibrium. Although aminoglycosides are a group of antibiotics that cause ototoxicity, they are still frequently used because of their effectiveness and low cost (Roland and Cohen, 1998).
Gentamicin is a positively charged aminoglycoside antibiotic that is selectively toxic to inner ear sensory hair cells (Miller, 1985). Gentamicin is used clinically to treat life-threatening, Gram-negative bacterial infections (e.g., meningitis), to prevent infection in patients with severe burns or large wound injuries, and also in premature babies. However, gentamicin ototoxicity remains a serious clinical (Hawkins et al., 1969; Wersall et al., 1969).

The results of the present study showed that all samples were under the Detection Limit indicating that gentamicin and polymyxin B sulphate at the used concentration could not be absorbed. Additionally, there were no difference between the skin at the umbilical abdominal region and inner ear skin. These data suggest that once tropical application of aminoglycosides for more than three hours has a predictably lower probability of systemic absorption and the probability of causing ototoxicity is reduced.

In conclusion, results have shown that tropical application of aminoglycoside, when applied to animal with a normal healthy skin, is less toxic. However, the potential for lowering the risk of ototoxicity from aminoglycosides is important and should be further investigated. As long as aminoglycoside is at least as efficacious and it clearly brings benefit to the patient in terms of toxicity avoidance. Furthermore,
using as little of the drug for as short a course as possible may reduce the risks of this complication developing.

REFERENCES


