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Determination of a dosage regimen of colistin by pharmacokinetic/pharmacodynamic integration and modeling for treatment of G.I.T. disease in pigs

J. Guyonnet [a,*], B. Manco [a], L. Baduel [a], V. Kaltsatos [a], M.H.F.S. Aliabadi [b], P. Lees [c]

[a] CEVA Santé Animale, Preclinical Department, La Ballastiere, BP 126 - 33501 Libourne Cedex, France
[b] Faroogh Life Sciences Research Laboratory, 122, Parcham St., Tohid Square, 1457844393 Tehran, Iran
[c] The Royal Veterinary College, Department of Veterinary Basic Sciences, Hawkshead Campus, Hatfield, Herts AL9 4TA, UK

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ABSTRACT

Colistin is an antimicrobial drug of the polymyxin group and COLIVET SOLUTION is an aqueous solution containing colistin sulphate (2×10^6 IU/mL), formulated for oral administration. The target species is the pig, particularly the suckling and post weaning animal. This investigation was undertaken to provide pharmacokinetic and pharmacodynamic data on which to base the selection of dosage rate and interval of the solution for the treatment of porcine colibacillosis.

Colistin absorption from the gastrointestinal tract of young pigs, when administered at dosage rates of 25,000, 50,000 and 1,00,000 IU/kg, was slight or absent. The drug was therefore restricted almost entirely to the required site of action. The colistin concentration–time profile within the jejunum and ileum was established, and this enabled determination of the pharmacokinetic variables, maximum concentration (C_{max}) and area under curve (AUC) and derivation of the surrogate indices of antibacterial activity, C_{max}/minimum inhibitory concentration (MIC) and AUC/MIC through integration of *in vivo* data with the results of *in vitro* potency studies for four strains of *Escherichia coli*.

In the *in vitro* bacterial growth inhibition studies colistin acted by a concentration-dependent killing mechanism. Numerical values for the surrogate parameter AUC/MIC producing bactericidal and eradication effects of colistin against four strains of *E. coli* were established by PK–PD modeling based on the sigmoidal E_{max} equation. These data were used to predict a daily dosage regimen for colistin.

1. Introduction

Colistin is an antibiotic of the polymyxin group produced by *Bacillus polymyxa var. colistinus.* It was discovered by Koyama et al. (1950). The polymyxins are cyclic lipopeptides; Chemically, colistin is a strong organic base, used clinically in the form of its water soluble sulphate and methanesulphonate salts. It is a bactericide with activity against gram negative enterobacteria and possessing a rapid onset of action. Its mechanism of action is similar to that of surface active agents, resulting in actions at two levels: (a) at low concentrations disorganisation of the cell membrane leading to leakage of intracellular contents; and (b) at higher concentrations inhibition of oxidative metabolism. Colistin also exerts an anti-endoxin effect through direct interaction with endotoxins. It is recommended for the therapy of gastrointestinal tract (g.i.t.) infections in the pig, particularly those caused by *Escherichia coli* (Nakajima and Kawamata, 1965; Greenwood, 1975; Rogers and Cohen, 1986; Evans et al., 1999).

Colistin has been used extensively in the livestock industry. Clinical use of the drug in pigs over a period of 20 years, at a dosage of 50,000 IU/kg every 12 h for 5 days, has provided evidence of excellent efficacy under field conditions (Belloc et al., 2008; Chauvin et al., 2002). In particular, colistin is commonly administered by the oral route in pigs for the treatment of colibacillosis (Nakajima and Kawamata, 1965; Casal et al., 2007). A similar use of colistin in the therapy of human g.i.t. bacterial infections has been reported (Rogers and Cohen, 1986). The susceptibility to colistin of the main enteropathogenic bacteria responsible for porcine g.i.t. infections and the fact that colistin is not absorbed from the gastrointestinal tract (Sato and Koumi, 1972) are important criteria in the selection of colistin for the treatment of g.i.t. infections.

The selection of dose rate and dose interval involves the additional consideration of a posology which not only ensures effectiveness against susceptible pathogens but which also minimises the emergence of resistance (Lees and Aliabadi, 2002; Drusano, 2004; Lees et al., 2004; Mouton, 2005). However, the conventional approaches to dosage schedule determination, including dose titration and dose confirmation studies (which usually monitor solely clinical efficacy), generally do not take into account the second but not secondary objective of limiting the emergence and spread

* Corresponding author. Tel.: +33 (0) 5 57 55 42 19; fax: +33 (0) 5 57 55 41 96.
E-mail address: guyonnet@ceva.com (J. Guyonnet).

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of resistance. Alternative approaches must therefore be investigated, established and then validated. The use of pre-clinical data based on pharmacokinetic–pharmacodynamic (PK–PD) modeling either to replace or to complement dose-titration studies has recently been proposed (Lees and Aliabadi, 2002; Lees et al., 2004; Toutain, 2002; Toutain and Lees, 2004; Toutain et al., 2004; Toutain et al., 2007).

The objectives of this study were: (a) to determine the concentration–time course of colistin in plasma and within the g.i.t. in healthy pigs after oral dosing of the drug at three dose rates; (b) to derive pharmacokinetic variables describing plasma and g.i.t. disposition; (c) to determine *in vitro* the time course of colistin induced inhibition of growth of pathogenic strains of *E. coli*; (d) to derive by PK–PD integration surrogate parameters of antimicrobial efficacy; and (e) to explore the value of a pre-clinical PK–PD modeling approach to determination of a colistin dose for pigs. The optimal colistin dose for treatment of colibacillosis pigs was assumed to be that which, with short term dosing, eradicates the pathogen and thus minimises the risk of emergence and spread of resistance with avoidance of the additional consequence of therapeutic failures.

2. Materials and methods

2.1. In vitro growth inhibition assay

An *in vitro* investigation was undertaken to determine the rate and extent of bacterial killing by colistin against four potentially pathogenic *E. coli* strains of piglet diarrhoea origin. *E. coli* isolates were obtained between 1998 and 2001 from clinical studies conducted under field conditions (UK, France, Germany, Spain, Belgium). The time-kill curves were established by measurement of bacterial count on serial samples taken from culture media containing the organisms in the presence of a range of concentrations of colistin. For the four *E. coli* strains isolated from porcine faeces (serotypes O101, O9, K88 (now known as F4) and not serotypable strain), minimum inhibitory concentrations (MICs) were determined by the broth dilution method. To establish growth inhibition curves, an inoculum of each strain containing approximately 10^6 cfu/mL was sub-cultured from beads into Mueller Hinton broth. Cultures were incubated under aerobic conditions at 35 °C for 24 h in the presence of increasing concentrations of colistin, expressed as multiples of MIC (0.5, 1, 2, 4, 8, 16 and 32 × MIC). Working standards of colistin (as the sulphate salt) were prepared in sterile purified water. Using 0.5 mL of each standard and 4.5 mL Mueller Hinton broth, containing *E. coli* suspensions, the final suspensions were prepared for incubation. Final concentrations ranged from 0.25 to 16 μg/mL (for three strains of MIC = 0.5 μg/mL; O101, O9 and not serotypable) and 0.5–32 μg/mL (for a single strain of MIC = 1 μg/mL; K88). Bacterial count (cfu/mL) was measured after 1, 3, 5 and 24 h incubation, by re-seeding aliquots on agar medium in the absence of colistin.

2.2. Animals

A pharmacokinetic study was carried out in 33 healthy male fattening pigs, aged approximately 7 weeks and of mean (±SD) weight 15.8 ± 1.9 kg (range 13–19 kg) on Day –1 (D – 1). The animals were typical of those in which clinical use is intended. All were males and all were supplied from a single source. They were individually identified with numbered ear tags.

2.3. Product

The test product was a drinkable solution of colistin as the sulphate (Colivet Solution, 200D0, CEVA Santé Animale, Libourne, France). The colistin used to prepare the solution contained 20,910 IU/mg colistin sulphate. The test product was formulated for oral administration and contained colistin at a concentration of 2.07×10^6 IU/mL.

2.4. Experimental design

The pigs were maintained in temperature controlled facilities in pens, with 3–4 animals per pen and observed daily for any signs of ill health. They had free access to water which was weighed and supplied once daily. They were fed a pelleted antibiotic-free commercial ration *ad libitum*, which was weighed to determine intake and also provided once daily. Food was withheld for 12 h before dosing and a small feed ration was given on the morning of D0. Animals were randomly allocated to one of three dosage groups, each containing 11 pigs, on the basis of body weight.

The test product was administered orally as a single dose on Day 0. The dose levels investigated were 25,000, 50,000 and 1,00,000 IU/kg (corresponding to 1.2, 2.4, and 4.8 mg/kg). One mL of the test product was diluted in drinking water and the appropriate volume administered according to body weight.

2.5. Sampling procedure

Blood samples (10 mL) from the anterior vena cava were collected into EDTA at the following slaughter times: 30 min and 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 h (one pig per time) after dosing. Plasma was separated by centrifugation and stored in the dark at approximately −80 °C prior to analysis. Gastrointestinal tract digesta samples (obtained from the duodenum to ileum and including g.i.t. wall and content) were collected at necropsy as a single sample at the following times (one animal per slaughter time): just prior to treatment and at 30 min and 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after dosing. Samples were collected immediately after killing and homogenized within 1 h of sampling.

2.6. Analytical methods

Colistin concentrations in plasma (0.5 mL) and g.i.t. contents (1.0 g) were determined using a validated high pressure liquid chromatography (HPLC) assay. Colistin does not contain a functional group that can be readily detected by HPLC detectors. Therefore, pre-column derivatization was undertaken to form a compound which could be detected by fluorescence (the derivatization reagent was a mixture of O-phthaldialdehyde with pH 10.5 orthoboric acid buffer and mercaptoethanol). In samples of 0.5 mL plasma and 1 g g.i.t. contents, protein was precipitated using trichloroacetic acid. This was followed by solid phase extraction and derivatization, in which 0.2 mL of derivatization agent was added to the sample vial by the autosampler. The sample was mixed for 1 min before injection onto the column. Colistin was separated on a C4 reversed phase column, with detection by fluorescence at an excitation wavelength of 340 nm and emission wavelength of 440 nm. This method determined total colistin and was validated for precision (range 5.32–7.81%), accuracy (range 5.9 to + 4.1) and recovery (range 73.6–82.9%). Quantitation was achieved by interpolation of response against concentrations on the calibration curve. The lower limits of quantitation (LLOQ) of the method were 0.25 μg/mL of plasma and 0.5 μg/g of gut contents.

2.7. Pharmacokinetic analyses

Pharmacokinetic analyses of colistin g.i.t. concentrations were performed using a program for non-linear regression analysis; KINETICA version 3.0 computer program (Thermo Electron, USA), a Microsoft-Windows based software program designed for non-compartmental modeling.