DOSE DETERMINATION FOR BACITRACIN AGAINST *CLOSTRIDIUM PERFRINGENS* USING A PHARMACOKINETIC/ PHARMACODYNAMIC (PK/PD) APPROACH. IMPACT ON CONSUMER SAFETY

Richez P.[1]*, Richard A.[2], Cornez B.[3], Vancraeynest D.[3]

[1]TransPharm, BP 7, 34160 St Geniès des Mourgues, France
[2]Alpharma, 3 Impasse de la Noisette, 91374 Verrière le Buisson, France
[3]Alpharma, Laarstraat 16, 2610 Antwerp, Belgium
*Corresponding author: pascal.richez@transpharm.fr

ABSTRACT

Bacitracin was administered continuously in the drinking water to four groups of growing rabbits at doses of 105, 210, 420 or 840 IU/kg body weight as a water-soluble veterinary medicinal product (Bacivet®-S) for seven consecutive days. Cecum samples were taken 0, 4, 8, 12 and 24 hours post-dosing on days 3 and 7 of the treatment. These samples were analysed using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS). The results show that 105 IU/kg did not expose the cecum to inhibitory concentrations and 210 IU/kg failed to maintain bactericidal levels for more than a few hours. The 420 IU/mg dose maintained bactericidal concentrations for the entire 24-hour period with 2102 µg/kg as the lowest mean concentration measured over the considered period, a value close to the target Minimal Bactericidal Concentration of 1860 µg/ml estimated for bacitracin *in vitro*. Concentrations obtained at 840 IU/kg were far higher. These results justify a daily dose of 420 IU/kg/day in the treatment of intestinal infections where *Clostridium perfringens* can be isolated. In addition, after 30 days of continuous administration at 420 IU/kg body weight, blood concentrations remained below the detection limit (<37.5 µg/l). As a consequence, no residues were found (<75 µg/kg) in any edible tissue from the first day after treatment withdrawal.

Key words: Rabbit, Bacitracin, Pharmacokinetics, PK/PD, Epizootic Rabbit Enteropathy, *Clostridium perfringens*.

INTRODUCTION

The veterinary medicinal product BACIVET®-S was recently licensed in France. This water-soluble form contains bacitracin zinc (4,200 IU/g) as active substance and is indicated in growing rabbits to reduce the clinical signs and mortality where strains of *Clostridium perfringens* susceptible to bacitracin can be isolated. The treatment should be instigated if the facility has previously suffered outbreaks and as soon as the first deaths are confirmed. Bacitracin is a polypeptide antibiotic made up of a mixture of several closely related polypeptides. It interferes with bacterial cell wall biosynthesis by inhibiting the pyrophosphatase involved in transporting peptidoglycan precursors through membranes (EMEA, 2001; Chambers, 2001). The zinc salt ensures that the active substance remains stable during storage. Bacitracin is bactericidal against Gram-positive cocci and bacilli, particularly against certain strains of clostridia (Jawatz, 1995). The aim of this *in vivo* study was to determine (i) the pharmacokinetic profile of bacitracin in the cecum to predict the optimum dosage regimen on a pharmacokinetic/pharmacodynamic basis, (ii) bacitracin systemic bioavailability (penetration into the blood compartment) and (iii) tissue residues after continuous administration of bacitracin as a water-soluble form to healthy rabbits in drinking water.

MATERIALS AND METHODS

A total of 88 New Zealand White rabbits (44 males and 44 females) aged 5 weeks received bacitracin zinc continuously in drinking water (Bacivet®-S, Alpharma, Verrière le Buisson, F-91374) for 7 consecutive days at four dose levels: 105, 210, 420 and 840 UI/kg body weight/day (1.7, 3.4, 6.8 and 13.5 mg bacitracin/kg body weight on the basis of a 62 IU/mg potency for the batch used). Concentrations in drinking water were adjusted daily on the basis of water intake on the previous day. Repeated assays of drinking water samples confirmed the accuracy of the doses administered (±10%). The rabbits were humanely killed by groups of 4 on the third and seventh days of the study at T0 (8.00 a.m.), +4h, +8h, +12h and +24h after the start of medicated water distribution. Bacitracin (free fraction) was assayed in the cecal content of each rabbit using a novel technique based on Liquid Chromatography with tandem Mass Spectrometry detection (positive mode) after ionisation using an Electro-Spray Interface (LC-MS/MS-ESI). Bacitracin was extracted from the gastro-intestinal tract into methanol/water/acetic acid (90/9/1); the supernatant was centrifuged then diluted with water and acidified prior to analysis. Quantities were expressed as total free bacitracin including the sum of the two main peaks (bacitracin A and B), on the basis of a bacitracin zinc batch with a potency of 62 IU/mg (dry substance).

Sixteen other rabbits, 8 males and 8 females, then received bacitracin zinc 420 IU/kg body weight orally in drinking water as Bacivet®-S for 30 consecutive days. Bacitracin concentrations were assayed in plasma, kidneys, liver, muscle and abdominal fat in 4 rabbits per sampling time point: 24, 48, 72 and 96 hours post-treatment withdrawal. Bacitracin was assayed using a similar method (LC-MS/MS-ESI) to that described above for cecal contents. This technique was validated with a quantification limit of 37.5 µg/kg (plasma) to 75 µg/kg (edible tissues).

Individual cecal concentrations were used at each sampling time point to determine the confidence interval (P=0.95). The efficacy criterion (surrogate) was the ability of the treatment to maintain bactericidal concentrations throughout the entire treatment period. Mean concentrations obtained in each group and at each time point were compared by Analysis of Variance (ANOVA) followed by a multiple post-ANOVA comparison test (Newman-Keuls test) for purposes of comparisons between the four groups at any sampling time point and between time points for any treated group.

RESULTS

The analytical techniques used in this study were validated on the basis of the following criteria: selectivity for bacitracin vs. other matrix components, linearity over the assay range including the limit of quantification (37.5 µg/kg in plasma, 75 µg/kg in edible tissues and 200 µg/kg in cecal content), precision (RSD for repeatability/reproducibility remained <15%), accuracy (recovery included 100% in the 95% confidence interval). Mean bacitracin concentrations in cecum are shown in Table 1. As the values obtained on Days 3 and 7 were not statistically different (P>0.05), mean values were obtained by pooling the data.

Table 1: Mean bacitracin concentrations (± SEM) at different sampling time points (pooled data Day 3 and Day 7, n = 8) as µg/kg of cecal content

Dose/day	105 IU/kg		210 IU/kg		420 IU/kg		840 IU/kg	
Time (h)	M	SEM	M	SEM	M	SEM	M	SEM
0	205.75	58.3072	661.5	104.9591	2731.25	872.335	7332.5	358.1521
4	520.5	195.1139	1582.5	483.5074	7062.5	2414.534	16290	3269.725
8	563.25	63.81402	2192.5	500.2562	7982.5	1019.137	20772.5	3269.086
12	418.5	60.85844	1905	133.5727	6807.5	1756.86	22975	3597.54
24	343.25	42.21448	869.75	54.70889	2102	530.4313	9545	2999.724

M = mean value (n = 8), SEM = Standard Error of the Mean (n = 8)

These results are shown in Figure 1 where threshold values are given for Minimal Inhibitory Concentrations (MIC_{90}) and Minimal Bactericidal Concentrations (MBC_{90}), corresponding to 930 and 1860 µg/kg, respectively (Richez *et al.*, 2008). The mean curve follows nycthemeral changes with a plateau observed between +4 and +12 hours which corresponds to the diurnal period. The lowest concentrations were recorded during the nocturnal phase, probably related to a lower water intake during the night.

The 18630 µg/kg MBC_{90} threshold could not be maintained by dosing at 105 or 210 IU/kg body weight whereas 420 IU/kg body weight maintained cecal concentrations above this threshold for the entire period, including night's end (0–24 h). The dose corresponding to 840 UI/kg body weight resulted in continuous supra-bactericidal levels with mean levels approximately 2 to 4 fold the MBC_{90}.

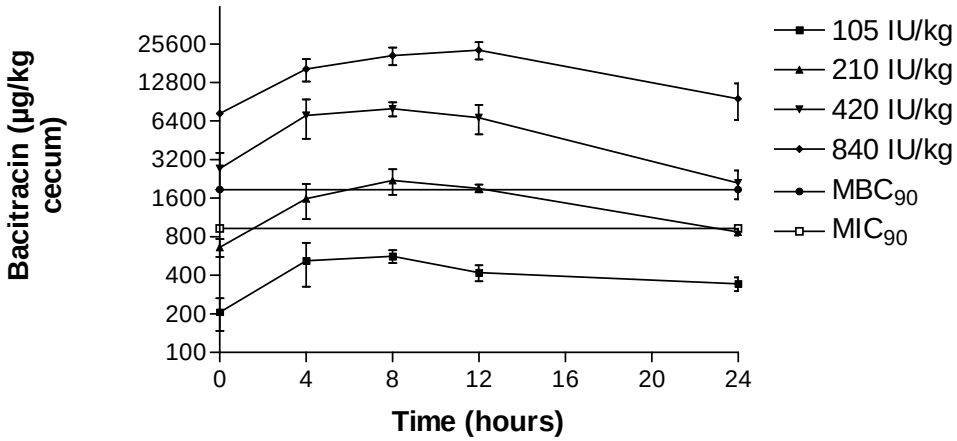

Figure 1: Mean bacitracin concentrations (± S.E.M.) in cecum at various sampling time points (pooled values Day 3 and Day 7, n = 8), µg/kg of cecal content, at 4 dose levels ranging from 105 to 840 IU/kg of body weight

The analyses performed after 30 days of continuous administration at 420 IU/kg body weight failed to detect any bacitracin in the plasma, thus showing that bacitracin bioavailability is virtually nil after oral ingestion (the technique's quantification limit was 37.5 µg/l). No measurable bacitracin was detected in any tissues sampled after treatment withdrawal (quantification limit was 75 µg/kg).

DISCUSSION

The results obtained showed that virtually no bacitracin was absorbed after continuous oral ingestion over a 30-day period despite the assay technique's very low limit of quantification in plasma (37.5 µg/l). These results are consisted with previous findings in pigs, chickens and rats (Butaye *et al.*, 2003; Froyshov *et al.*, 1986). This apparent lack of absorption can, at least partly, be explained by the macromolecular properties of bacitracin, a polypeptide with a molecular weight of 1422 daltons for the bacitracin A fraction, and by its physico-chemical properties: high water solubility, weak lipophilic profile, pKa of 5.5 leading to a high degree if ionisation at the pH reached in the distal part of the intestinal tract (pH 6 to 8 on average). In consequence, bacitracin undergoes ion trapping in the intestine that results in its non-absorption and maintenance of relatively constant concentrations in the digestive tract lumen. As a result, bacitracin is very unlikely to undergo parietal transfer. This virtually zero systemic bioavailability leads to undetectable amounts of residues in the edible tissues (muscle, liver, kidneys, fat) from the first sampling time point (+24 h post-withdrawal) onward. All levels thus remained far below the Maximum Residue Limits (MRLs) as defined by the European Commission (EC Council Regulation 2377/90, 2003 has established MRLs of 150 µg/kg in edible tissues).

Any determination of an optimal therapeutic regimen for bacitracin must consider the pharmacodynamic properties of this antibiotic. Previous studies (Richez *et al.*, 2008) revealed that

bacitracin is a bactericidal antibiotic of the concentration-independent type (i.e. time-dependent). This pharmacodynamic profile implies that bactericidal levels should be maintained constantly at the infection site throughout the entire treatment period (McKellar *et al.*, 2004). Pharmacokinetic data obtained in the present study established that MBC_{90} levels, i.e. 1860 IU/kg, should thus be maintained in the cecal lumen (site of infection where *Clostridium perfringens* can be present). The calculation of an optimal dosing regimen on the basis of pharmacokinetic/pharmacodynamic data (PK/PD) in the light of the results obtained in this study led us to conclude that 420 IU/kg body weight (theoretically) represents this optimum level.

CONCLUSIONS

The results obtained in this study made it possible to calculate an optimal dosing regimen on the basis of pharmacokinetic/pharmacodynamic data. A daily bacitracin dose of 420 UI/kg in the form of Bacivet®-S maintained cecal concentrations at least equal to the MBC_{90} throughout the entire treatment period. But, treatment duration is not a parameter that can be derived from PK/PD analyses and must therefore be determined from clinical field studies, outside the scope of the present research programme. Bacivet®-S has been approved in Europe - on the basis of additional clinical trials - for the control of infections where *C. perfringens* can be isolated, at a dose of 420 IU/kg body weight when given in drinking water for 14 to 21 days. Prolonged administration over 30 days failed to result in measurable amounts of bacitracin in blood due to virtually zero systemic bioavailability after oral ingestion. As a consequence, all tissue concentrations remained far below the Maximum Residue Limits, thus ensuring consumer safety even with very short withdrawal periods.

ACKNOWLEDGEMENTS

Our thanks go to Mark H. Jones for his editorial assistance and suggestions to improve the paper.

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