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Pharmacokinetic–pharmacodynamic integration and modelling of oxytetracycline administered alone and in combination with carprofen in calves

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ABSTRACT

The pharmacokinetic (PK) and pharmacodynamic (PD) profiles of oxytetracycline were investigated, when administered both alone and in the presence of carprofen, in healthy calves. The study comprised a four treatment, four sequences, and four period cross-over design and used a tissue cage model, which permitted the collection of serum, inflamed tissue cage fluid (exudate) and non-inflamed tissue cage fluid (transudate). There were no clinically relevant differences in the PK profile of oxytetracycline when administered alone and when administered with carprofen. PK–PD integration was undertaken for a pathogenic strain of *Mannheimia haemolytic* (A1 76/1), by correlating *in vitro* minimum inhibitory concentration (MIC) and time-kill data with *in vivo* PK data obtained in the cross-over study. Based on *in vitro* susceptibility in cation adjusted Mueller Hinton Broth (CAMHB) and *in vivo* determined PK variables, ratios of maximum concentration (C_{max}) and area under curve (AUC) to MIC and time for which concentration exceeded MIC ($T > MIC$) were determined. The CAMHB MIC data satisfied integrated PK/PD relationships predicted to achieve efficacy for approximately 48 h after dosing; mean values for serum were 5.13 (C_{max}/MIC), 49.3 h ($T > MIC$) and 126.6 h (AUC_{96h}/MIC). Similar findings were obtained when oxytetracycline was administered in the presence of carprofen, with PK–PD indices based on MIC determined in CAMHB. However, PK–PD integration of data, based on oxytetracycline MICs determined in the biological fluids, serum, exudate and transudate, suggest that it possesses, at most, limited direct killing activity against the *M. haemolytica* strain A1 76/1; mean values for serum were 0.277 (C_{max}/MIC), 0 h ($T > MIC$) and 6.84 h (AUC_{96h}/MIC). The data suggest that the beneficial therapeutic effects of oxytetracycline may depend, at least in part, on actions other than direct inhibition of bacterial growth.

1. Introduction

The rationale for administering non-steroidal anti-inflammatory drugs (NSAIDs), as adjuncts to antimicrobial drug treatment of calf pneumonia, is based on the excessive inflammatory response of the host defense mechanisms within the lungs. This response involves the release of many inflammatory mediators leading to pulmonary oedema (Deleforge et al., 1994; Elitok and Elitok, 2004). Two drugs used extensively to treat bovine respiratory disease are oxytetracycline and carprofen. As both are marketed by the same manufacturer, they are frequently co-administered in clinical use.

Oxytetracycline is a broad spectrum antimicrobial agent with activity against many pathogens, including *Mannheimia haemolytic*, a causative organism of bovine pneumonia (Nouws et al., 1985a,b, 1990; Toutain and Raynaud, 1983). It is classified as a bacteriostatic agent, but *in vitro* bactericidal actions can be readily demonstrated. Licensed 20% and 30% formulations of oxytetracycline have persistent actions, because of the high strength and high dosage used (20 or 30 mg/kg), leading to sustained absorption from the reservoir site of intramuscular injection. This gives rise to flip-flop pharmacokinetics (PK) (Nouws and Vree, 1983; Nouws et al., 1990; Toutain and Raynaud, 1983).

Carprofen is a NSAID which is well tolerated in cattle (Ludwig et al., 1989; Lohuis et al., 1991; Vangroenweghe et al., 2005). Its anti-inflammatory, antipyretic and analgesic properties provide the basis for its use in combination with antimicrobial drugs, in pneumonias of young calves (Lees et al., 1996). The latter group established the PK profiles of carprofen enantiomers in calves, but the dose rate of 0.7 mg/kg was lower than that now recommended of 1.4 mg/kg.

There are no published data to indicate whether and (if so) how oxytetracycline and carprofen interact. Knowledge of drug interactions is required to assure efficacy and safety and also to develop dosing schedules, which optimize both clinical and bacteriological cures, thereby minimising the emergence of resistance (Sidhu et al., 2005, 2010).

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PK interactions between antimicrobial drugs and NSAIDs have been described in cattle and goats (Mestorino et al., 2007; Sidhu et al., 2005, 2006, 2010) and pharmacodynamic (PD) interactions have also been reported (Dutta et al., 2007).

NSAIDs are widely used in human medicine and interactions with drugs of other classes, occasionally leading to increased plasma concentrations, are common (Verbeeck, 1990). Such interactions can arise through alteration to metabolism or excretion of the NSAIDs. Carprofen is highly bound to plasma proteins and therefore excreted in urine only in very small amounts as unchanged drug, as no more than 1% of plasma concentration is filtered at the glomerulus. Elimination from the body is primarily by glucuronidation in the liver in rat, man, dog and horse (Rubio et al., 1980; Ray and Wade, 1982; Soraci et al., 1995; Delatour et al., 1996). This phase II reaction is enantioselective, occurring predominantly for the *S*(+) enantiomer. Data are not available for this pathway in calves. Oxytetracycline is eliminated primarily as unchanged drug in the urine, by glomerular filtration, proximal tubular secretion and distal tubular reabsorption. In calves, almost 50% of the administered dose is excreted in urine within 12 h of dosing intravenously (Xia et al., 1983; Nouws et al., 1985a; Mevius et al., 1986). In addition, oxytetracycline is excreted in bile in some species (Serrano et al., 1999), but urinary excretion usually predominates.

Tetracyclines may cause dose dependent toxicosis of renal tubules which may be exacerbated by other nephroxtoxic drugs (Riond and Riviere, 1989). Although tetracyclines are not anionic, renal proximal tubular secretion and reabsorption does appear to be mediated by organic anion transporters (Babu et al., 2002) and this may contribute to tetracycline-induced nephrotoxicity. It may also affect the PK and/or toxicosis of co-administered drugs, which are transported by organic anion transporters in the kidney or which modulate the effects of these transporters. Several NSAIDs inhibit organic anion transporters (Masuda et al., 1997) and this has been demonstrated to increase the systemic exposure of tetracycline co-administered with naproxen or diclofenac in rats (Oh and Han, 2006). The latter authors demonstrated that renal clearance of tetracycline was reduced approximately threefold in the presence of naproxen or diclofenac. The systemic exposures to tetracycline in the rats pretreated with naproxen or diclofenac were significantly higher than those from a control group given tetracycline alone. Mean terminal plasma half-life of tetracycline was increased by two- to fivefold with pretreatment with naproxen or diclofenac. The authors suggested that NSAIDs such as naproxen and diclofenac may alter the renal elimination and PK profile of tetracycline.

Given the known interactions of some tetracyclines and NSAIDs, and their glucuronide phase II metabolites, on renal tubular organic anion transporters and, since oxytetracycline is known to be excreted predominantly by the kidney, this could be a mechanism whereby the PK of oxytetracycline could be affected by co-administration with carprofen. However, the low renal excretion of carprofen makes this unlikely. Carprofen is metabolised slowly in cattle and is largely excreted unchanged (EMEA, 1999). However, metabolism by ester glucuronidation is important in other species (Rubio et al., 1980) and is likely to occur but to an unknown extent in cattle. Glucuronidation of the weak anti-inflammatory drug acetaminophen is known to be significantly inhibited by tetracycline (Bolanowska and Gessner, 1978), suggesting that in some species metabolic interactions between NSAIDs and oxytetracycline could occur and thereby affect the PK of either drug. Another possibility is competition between drugs for binding to plasma proteins.

PD interactions have also been described for some NSAIDs used in combination with antimicrobial drugs. Diclofenac has been shown to have a synergistic antibacterial effect on *Mycobacterium tuberculosis*, when used in combination with streptomycin (Dutta et al., 2007). Furthermore, oxytetracycline has been shown to have anti-inflammatory activity independent of its antimicrobial action (Ci et al., 2011) and clearly this could confer an additive or synergistic anti-inflammatory effect when used in combination with a NSAID.

The potential PK, metabolic and PD interactions of antimicrobial drugs and NSAIDs are poorly understood and when such drugs are commonly used in combination these interactions deserve investigation.

There are no literature reports describing the PK of oxytetracycline when administered in combination with carprofen in calves. Therefore, the present investigation was undertaken with the overall aim of investigating interactions between these drugs in healthy calves with the following objectives: (1) to determine serum, exudate and transudate concentration–time relationships and derive PK variables for oxytetracycline following intramuscular administration at the dose rate of 20 mg/kg; (2) to establish PK data for oxytetracycline after co-administration with carprofen, injected subcutaneously at the recommended dose rate of 1.4 mg/kg; (3) to generate PD data for oxytetracycline against a pathogenic strain of *M. haemolytica*; and (4) to integrate and model PK and PD data for oxytetracycline, as a basis for dosage prediction in the absence and presence of carprofen.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Royal Veterinary College Ethics and Welfare Committee and was carried out under the United Kingdom's Animal Scientific Procedures Act.

2.2. Animals and tissue cage model

The study was conducted in eight healthy male calves, weighing 110–135 kg and approximately 4 months old at study commencement. Each animal was housed individually in a ventilated barn and provided with free access to hay, water and an antibiotic-free concentrate ration. Tissue cages were prepared and inserted subcutaneously, three each in the right and left flank regions, under general anaesthesia as described by Sidhu et al. (2003). The animals were allowed to recover from surgery for 5 weeks to permit wound healing and the growth of granulation tissue encapsulating and into the cages.

2.3. Experimental design and model of inflammation

A four period, four sequences, four treatment cross-over Latin square design was used, such that each animal received each treatment in sequence. Calves were allocated to the four treatment groups, with two animals receiving each treatment in each period. An injectable long acting veterinary formulation of oxytetracycline hydrochloride (Terramycin™-20%, Pfizer Ltd., Sandwich, Kent, UK) and carprofen (Rimadyl Large Animal Solution containing 50 mg/mL carprofen, Pfizer Ltd., UK) were supplied by the manufacturer. The four treatments, administered at zero time, were: oxytetracycline, carprofen, oxytetracycline plus carprofen and placebo. The placebo injection was sterile normal saline, administered intramuscularly in a dose volume equivalent to 20 mg/kg oxytetracycline. Intervals of 14/15 days were allowed between each period.

Oxytetracycline was administered intramuscularly at a dose rate of 20 mg/kg, each calf receiving two equal volume injections into opposite gluteal muscles. Carprofen was administered subcutaneously at a single site at a dose rate of 1.4 mg/kg into the neck. At zero time, in each period, an intra-caveal injection of 0.5 mL of