Theses of doctoral (PhD) dissertation

Pharmacokinetic and pharmacodynamic investigation of florfenicol and enrofloxacin in pig synovial fluid and plasma

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Table of contents

1. Introduction and aims of research

One of the most important concerns affecting the health of human population today is antimicrobial resistance. One of the possible causes is to be found in animal health, where, during widespread antimicrobial use, pathogenic and commensal bacteria are becoming resistant to certain antibacterial agents in ever increasing proportions. The severity of the situation is highlighted in a report published by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA), that describes the use of antibacterial treatments in veterinary medicine and their dire consequences for public health. One of the key messages is that the use of antimicrobials in veterinary medicine should be significantly reduced, and/or should be replaced by alternative products (e.g. probiotics, prebiotics, herbal extracts) as far as possible, and that these valuable agents should only be used for therapy in justified cases supported by appropriate susceptibility testing.

When antibacterial agents are used in veterinary medicine, the exact dosage of the treatment associated with the different diseases, the interval between two administrations and the duration of administration are of key importance. The active substances florfenicol and enrofloxacin have a broad antibacterial spectrum and are used in a wide range of veterinary applications. In order to maintain their efficacy for as long as possible, their prudent use is of paramount importance. Florfenicol has been classified by the EMA as an AMEG Category C substance and therefore has an important role to play as a substitute for AMEG Category B substances highly important for human medicine. Enrofloxacin, as a Category B agent, and therefore should be reserved for those infections when there is no effective antibacterial agent in either category D or C and its use has been previously established with full certainty of efficacy. Prudent use is a priority for practising veterinarians and their work in this area needs to be supported by scientific proof. This objective is served by extensive studies on the pharmacokinetic and pharmacodynamic parameters of antibacterial agents. On the basis of these new results, new therapeutic proposals should be issued, where possible.

In the field of both pharmacokinetics and pharmacodynamics, one of the most important questions is the concentration of antibacterial agents at the site of action (site of infection), how the bacterial exposure to the agent occurs, and what response is expected from the bacterial populations causing the infection, i.e. the direction in which the medium in which the exposure occurs influences the efficacy of antibacterial agents.

The aim of the present work is to obtain more data on the pharmacokinetic properties of florfenicol and enrofloxacin, very commonly used in pig health, i.e. to determine their specific pharmacokinetic parameters at the site of action. In our study we investigated the distribution of the active substances in the synovial fluid in order to propose florfenicol and enrofloxacin as antibacterial therapy for joint diseases. The selected bacterial pathogen is *Streptococcus* (*S.*) *suis*, which is a major economic issue for the pig sector and is a particularly common cause of both acute and chronic arthritis in pigs, most commonly at 4-10 weeks of age.

Our aim was to investigate the pharmacokinetics of florfenicol in pig plasma and synovial fluid following intramuscular (im.) administration at the authorised dose of 15 mg/kg, and a higher dose of 30 mg/kg, following im. administration. For enrofloxacin, the pharmacokinetic properties of the active substance in pig plasma and synovial fluid were investigated at the authorised subcutaneous dose of 7.5 mg/kg. In all three cases, following the nonlinear pharmacokinetic analysis and the establishment of concentration-time curves, the pharmacokinetic properties of the active substances were evaluated at the population level using a nonlinear mixed-effects model (NLME) with single-compartment pharmacokinetic analysis in pigs at the in vivo study sites. The use of NLME was chosen to obtain more robust data for subsequent Monte Carlo simulation and thus to have the most accurate data available for an artificially constructed population. That is, after selecting the appropriate population pharmacokinetic models, we generated a hypothetical population of a large number of piglets using Monte Carlo simulation and used these to test the antibacterial activity of the active substances for florfenicol with regard to our pharmacodynamic targets (PDT) and for enrofloxacin with regard to the PDT found in the literature. For florfenicol and *S. suis*, our aim was to determine the PDT in synovial fluid modeling the site of infection, which was determined by plotting killing curves in three different media at different concentrations of florfenicol. With regard to population pharmacokinetic data and PDT values, we aimed to determine the pharmacokinetic/pharmacodynamic cut-off (PK/PD_{CO}) values of the active substances for *S. suis* in pig plasma and synovial fluid.

In addition, we aimed to determine the phenotypic antibiotic susceptibility of 100 clinical-origin *S. suis* isolates to florfenicol and enrofloxacin using a broth microdilution method and to calculate MIC_{50} and MIC_{90} values based on the MIC values. Furthermore, we determined the distribution of florfenicol and enrofloxacin susceptibility of *S. suis* isolates in Hungary according to the clinical breakpoint (CBP) defined by CLSI and recalculated these data based on our own PK/PD_{CO} values.

2. Overview of new scientific results

The present study was the first to investigate the pharmacokinetic properties of florfenicol at an authorised dose of 15 mg/kg and the higher dose of 30 mg/kg following intramuscular (im.) administration in pig synovial fluid, and the pharmacokinetic properties of enrofloxacin at an authorised dose of 7.5 mg/kg following subcutaneous (sc.) administration in pig synovial fluid. In addition, we determined for the first time the pharmacokinetic target of florfenicol in pig synovial fluid. As a result of this research work, we have proposed for the first time the pharmacokinetic/pharmacodynamic cut-off (PK/PD_{CO}) for florfenicol and enrofloxacin in swine arthritis caused by *S. suis*.

Main findings of the study are as follows:

1. The main population pharmacokinetic parameters (mean ± standard deviation) of florfenicol in pig synovial fluid following a single im. administration at a dose of 15 mg/kg:

T_{max} = 2.71 ± 1.32 h

C_{max} = 1.81 ± 0.82 µg/mL

Cl = 0.28 ± 0.12 (L/h)/kg

V_d = 9.18 ± 3.88 L/kg

$AUC_{0-\infty}$ = 64.69 ± 28.12 (h × µg)/mL

AUC_{0-24h} = 32.28 ± 13.91 (h × µg)/mL

t_{max} = time to peak synovial fluid concentration; C_{max} = maximum synovial fluid concentration; Cl = systemic clearance; V_d = volume of distribution; $AUC_{0-\infty}$ = area under the curve from zero time to infinity; AUC_{0-24h} = area under the curve for 24 h

2. The main population pharmacokinetic parameters (mean ± standard deviation) of florfenicol in pig synovial fluid following a single im. administration at a dose of 30 mg/kg:

T_{max} = 1.00 ± 0.00 h

C_{max} = 2.54 ± 0.67 µg/mL

Cl = 0.47 ± 0.16 (L/h)/kg

V_d = 12.45 ± 3.56 L/kg

$AUC_{0-\infty}$ = 71.42 ± 23.89 (h × µg)/mL

AUC_{0-24h} = 37.67 ± 4.64 (h × µg)/mL

t_{max} = time to peak synovial fluid concentration; C_{max} = maximum synovial fluid concentration; Cl = systemic clearance; V_d = volume of distribution; $AUC_{0-\infty}$ = area under the curve from zero time to infinity; AUC_{0-24h} = area under the curve for 24 h

3. The main population pharmacokinetic parameters (mean ± standard deviation) of enrofloxacin in pig synovial fluid following a single sc. administration at a dose of 7.5 mg/kg:

T_{max} = 8.6 ± 2.09 h

C_{max} = 1.67 ± 0.49 µg/mL

Cl = 0.18 ± 0.05 (L/h)/kg

V_d = 2.96 ± 0.97 L/kg

$AUC_{0-\infty}$ = 45.26 ± 12.68 (h × µg)/mL

AUC_{0-24h} = 30.30 ± 8.67 (h × µg)/mL

t_{max} = time to peak synovial fluid concentration; C_{max} = maximum synovial fluid concentration; Cl = systemic clearance; V_d = volume of distribution; $AUC_{0-\infty}$ = area under the curve from zero time to infinity; AUC_{0-24h} = area under the curve for 24 h

4. Pharmacodynamic targets of florfenicol in pig synovial fluid:

AUC_{24h}/MIC in bacteriostatic effect = 22 h

AUC_{24h}/MIC in bactericidal effect = 76 h

AUC_{24h}/MIC = the part of the time interval, expressed in hours, between two doses of the drug during which the concentration of the antibacterial agent exceeds the concentration required to inhibit bacterial growth

5. The PK/PD_{CO} value for florfenicol in the authorised dosage regimen (15 mg/kg, im.) for arthritis caused by *S. suis* was 0.5 µg/mL.

6. The PK/PD_{CO} value for the higher dosage regimen of florfenicol (30 mg/kg, im.) for arthritis caused by *S. suis* was 1 µg/mL.

7. The PK/PD_{CO} value for enrofloxacin in the authorised dosing regimen (7.5 mg/kg, sc.) for arthritis caused by *S. suis* was 0.5 µg/mL.

8. In this research, the MIC values of a total of 100 Hungarian *S. suis* isolates cultured from clinical samples were determined against florfenicol and enrofloxacin. Furthermore, MIC_{50} and MIC_{90} values were calculated, which were 2 and 8 µg/mL for florfenicol and 0.5 and 2 µg/mL for enrofloxacin, respectively.

Based on our results, florfenicol and enrofloxacin have potential therapeutic option for the treatment of arthritis caused by *S. suis*, which is associated with high economic losses in the swine industry. Furthermore, the study confirms the fact that, in parallel with ongoing antibiotic susceptibility testing, it is of paramount importance to study the pharmacokinetic and pharmacodynamic properties of antibacterial agents at the site of action.

3. Own scientific publications related to the topic of the dissertation

Full text papers in peer-reviewed journals

Somogyi Z., Karancsi Z., Jerzsele Á.: **Farmakokinetika/farmakodinámia (PK/PD) megközelítés az állatgyógyászatban**, Magyar Állatorvosok Lapja, 140. 37–46, 2018.

Somogyi Z., Mag P., Kovács D., Kerek Á., Szabó P., Makrai L., Jerzsele Á.: **Synovial and Systemic Pharmacokinetics of Florfenicol and PK/PD Integration against *Streptococcus suis* in Pigs**, Pharmaceutics, 14(1). 109, 2022.

Somogyi Z., Mag P., Simon R., Kerek Á., Szabó P., Albert E., Biksi I., Jerzsele Á.: **Pharmacokinetics and Pharmacodynamics of Florfenicol in Plasma and Synovial Fluid of Pigs at a Dose of 30 mg/kgbw Following Intramuscular Administration**, Antibiotics (Basel), 12(4) 758, 2023.

Somogyi Z., Mag P. Simon R., Kerek Á., Makrai L., Biksi I., Jerzsele Á.: **Susceptibility of *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Streptococcus suis* Isolated from Pigs in Hungary between 2018 and 2021**, Antibiotics (Basel), 12(8). 1298, 2023.