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Original article

Withdrawal of cefoperazone with milk after intramammary administration in dairy cows – prospective and retrospective analysis

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Abstract

The aim of the study was to carry out retrospective and prospective comparative analyses of the pharmacokinetics of CEF after single intramammary (IMM) administration in cows. The prospective study (study A) was conducted on 9 dairy cows of the Polish Black-White race with clinical mastitis during the lactation period. Milk samples were collected at 2, 4, 6, 8, 10, 24, 36, 48, 72 and 84 h after single IMM administration of 250 mg of CEF to one quarter. Drug concentrations in milk samples were determined by HPLC-MS/MS technique and the results of the pharmacokinetic analysis were compared to those obtained in previous studies based on the microbiological (study B) and HPLC-UV methods (study C and D). Pharmacokinetic parameters were calculated based on adapted two-compartment model of drug distribution. One of the findings of the comparison of the analysed investigations is that the CEF kinetics determined with the microbiological method is consistent with the results obtained by the authors of this paper. Both studies yielded similar results of the key pharmacokinetic parameters related to the level of the drug distribution to tissues and elimination half-life. In the pharmacodynamic analysis, the observations in all four studies were entirely consistent and have shown lower values of $T>MIC_{90}$ in healthy animals and significantly higher values in infected dairy cows. The comparison of studies A, B, C, and D revealed that the time of complete CEF wash-out of 90.90% varied and amounted to 5.7, 8.0, 2.2, and 2.2 days after administration of the drug, respectively.

It was confirmed that not only the type of the analytical method but also correct sampling have a significant impact on determination of the correct value of the drug half-life after IMM administration. The comparative analysis of studies in which the milk yield was high and low allows a conclusion that this parameter in the case of CEF has no significant effect on $T>MIC_{90}$.

Key words: pharmacokinetics, cefoperazone, mastitis, cows.

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Introduction

Inflammation of the mammary gland in cows is an important sanitary-epidemiological and economical issue. There is a number of veterinary products containing β-lactam antibiotics in their composition, which are used to control mastitis in cows (Bozhkova et al. 1983, Guerrini et al. 1985, Wilson et al. 1986, Novelli et al. 2000, Bradley et al. 2002) One of the β-lactam antibiotic groups are cephalosporins, among others cefoperazone (CEF) (Guerrini et al. 1985, Wilson et al. 1986, Sanders et al. 1988, Guterbock et al. 1993, Costa et al. 1998, Heringstad et al. 2000, Novelli et al. 2000, EC 2002, Błądek et al. 2011). Cephalosporins are commonly used in veterinary medicine due to their broad spectrum of activity when confronted with natural penicillins, low treatment cost, and safety.

Depending on their epidemiological link with the disease, bacteria that cause mastitis in cows are classified as contagious or environmental pathogens (Eenennaam et al. 1995, Watts et al. 1997, EMA 1998, Rossitoo et al. 2002). A principle in the treatment of mastitis is the introduction of therapeutic agents into udder quarters devoid of inflammatory secretions (Guterbock et al. 1993, Gruet et al. 2001, Malinowski et al. 2001).

An uncontrolled use of cephalosporins in animal treatment may result in antibiotic residues in food of animal origin (Guerrini et al. 1985, Wilson et al. 1986, Bradley et al. 2002, Novelli et al. 2002). However, the risks to human health are posed by antibiotic residues in milk produced by cattle receiving treatment of mammary gland inflammation (Bozhkova et al. 1983, Guerrini et al. 1985, Wilson et al. 1986, Bradley et al. 2002, Novelli et al. 2002, Burmańczuk et al. 2011). Due to the potential threat to human health, the European Union established maximum residue limits (MRLs) in milk for cefoperazone at 50 µg/kg level (EMA 1998). FDA did not establish tolerance or "safe levels" for CEF (FDA 2012).

To date, the kinetics of CEF elimination at intramammary (IMM) administration has been investigated in several papers. Only a few studies describe the kinetics of CEF wash-out. The published reports available currently have been based on bacteriological and HPLC-UV analyses (Wilson et al. 1986, Cagnardi et al. 2010).

Investigations of the kinetics of drugs administered via IMM route conducted by authors even several decades ago are not often cited or they are disregarded due to the considerable progress in the data analysis and bioanalytical methods. Comparison of results obtained with microbiological and HPLC-MS/MS methods is often disadvantageous for the former (Concordet et al. 1997, Daeseleire et al. 2000, Samanidou et al. 2003, De Brito et al. 2006). On the other hand, the current methods of data analysis allow analysing raw data obtained with older approaches, thereby restoring the high cognitive value of earlier research.

The aim of the present study was to determine the parameters of kinetics of CEF wash-out using the most modern analytical techniques (HPLC-MS/MS). Concurrently, a retrospective analysis of results obtained with the microbiological method was performed with the use of available software (Wilson et al. 1986). These results were also compared to the more comprehensive results provided by HPLC-UV (Cagnardi et al. 2010).

Materials and Methods

In this paper, prospective data (study A) and 3 groups of retrospective data (B, C, D) were analysed. In study B, the CEF concentration was analysed with the bacteriological method (Wilson et al. 1986), whereas study C (initial lactation phase) and D (late lactation phase) were based on the HPLC-UV methodology (Cagnardi et al. 2010). A description of the key elements differentiating the analyses is presented in Table 1.

Study A was carried out with the approval of the Local Ethical Committee in Lublin (Resolution No. 42/2009). The study was conducted on 9 dairy cows of the Polish Black-White race at the age of 4-10 years with clinical *mastitis* during the lactation period. The cows were examined in different lactation periods, i.e. from 1.5 month post-calving to the pre-drying period; the average milk yield in the cows was 25-30 L/24 h. The cows weighed approx. 650 kg each. They were qualified for the research on the basis of guidance from the owners and research exchanges. The cows were verified using a *mastitis* DRAMINSKI Mastitis Detector 4×4Q (Dramiński®, Poland) (a 4-quarter device for detecting milk resistance). Cows with milk resistance ≤ 130 Ω were qualified for the study. The animals were fed farming feed concentrates comprising commercial (wheat, rye) and fodder (oats, barley) grain alternating with raw corn and pasture grazing. Access to food and water was provided *ad libitum*.

The analyses were carried out between March and September. The cows came from different farms in the Lublin Province area. They received 10 mL of water solution containing 250 mg of CEF (Pathozone® 250 mg/10 mL, Pfizer®) to one quarter. Before the process of collection of milk samples, the teats were cleaned and immersed in a liquid disinfectant, Avitaderm (Agrovet – Śniadowo, Poland). Afterwards, their udders were wiped with a swab soaked in 70% ethanol. Subsequently, after single IMM administration, the milk samples (10 mL) were collected at the

Table 1. Key parameters and the design of the analysed studies.

Criterion/value	Study			
	A	B	C	D
Sample size	9	12	23	23
Strain	Black & White	Fresian	Italian Frisona	
Dose per quoter/total [mg]	250/250	250/1000	300/1200	
Status of quoters	Ma	H	Ma, H, S	Ma, H, S
Quoters treated	1		4	
Analytics	HPLC-MS/MS	microbiological	HPLC-UV	
LOQ [µg/mL]	0.002	0.010	0.010	
Milking	middle yielding	low yielding	initial period	late period
Milk yield [L/24h]	25-30	10	26-55	
	Pathozone® (Pfizer)			

A – original prospective study; B – study by Wilson and Gilbert (Wilson and Gilbert, 1986); C, D – study by Cagnardi et al. (2010); Ma – mastitis; H – healthy; S – suspected; HPLC-MS/MS – high performance liquid chromatography with mass spectrometry; HPLC-UV – high performance liquid chromatography with UV detection; LOQ – limit of quantification; nd. – no data.

following time points: 2, 4, 6, 8, 10, 24, 36, 48, 72 and 84 h. The milk samples were collected in dark plastic bottles and kept at -18ºC until analysis.

Bioanalytical method development and validation

The analytical method reported by Błądek et al. (2011) was used for determination of CEF in study A. Briefly, CEF was extracted from milk samples by acetonitrile and analysed on an Agilent 1200 Series LC system (Agilent Technologies, Santa Clara, USA) connected to an API 4000,, mass spectrometer (AB SCIEX, Ontario, Canada). Separation was achieved on a Luna® C18 reverse phase column (3 µm, 2.0 × 150 mm, Phenomenex®, Torrance, CA, USA) with the mobile phase consisting of acetonitrile and 0.025% heptafluorobutyric acid and elution in a gradient mode. Quantification was obtained using multiple-reaction monitoring (MRM) transition, which was selected as m/z 646→m/z 530 for CEF.

The method used in the present experiment was fully validated in accordance with current expectations (EC 2002). The following parameters were determined: linearity, specificity, selectivity, accuracy, precision (repeatability and within-laboratory reproducibility) and the decision limit (CCh) and detection capability (CCβ) were calculated. Additionally, the limit of detection (LOD) and the limit of quantification (LOQ) were established. Matrix-matched calibration curves were used for quantification with acceptable linearity (correlation coefficient, r > 0.999) over the range of 2-10000 µg/kg. The specificity of the method was checked by analysing 20 blank milk samples, and no peak was detected in these samples at the retention time corresponding to the analyte. The results of the selectivity studies indicated that none of the other cephalosporin antibiotics had an effect on the detector response intensity in the retention time of CEF. In the precision and accuracy study, blank milk samples were spiked with CEF at three different concentrations corresponding to 0.5×MRL, MRL, and 1.5×MRL, from run to run during 1 day and 3 days, respectively. Repeatability expressed as a coefficient of variation (CV%) was from 7.7 to 9.0%, and the within-laboratory reproducibility was from 10.1 to 11.7%. The overall mean concentrations obtained in the reproducibility study were used to calculate accuracy expressed as a percentage and varied from 96.2 to 99.4%. The CCh and CCβ were established at the level of 59 µg/kg and 71 µg/kg, respectively. The sensitivity of the method was satisfactory, which can be confirmed by the low LOD (1 µg/kg) and LOQ (2 µg/kg).

Pharmacokinetics analysis

The pharmacokinetic analyses were performed based on raw data using Phoenix® WinNonlin® 7.0 software (Certara L.P., US) and the statistical analyses were carried out with the GraphPad Prism® 6.01 program (GraphPad Software Inc., US). The calculations were based on the slope, height, area, and moment (SHAM) analysis and an adapted two-compartment model of drug distribution after IMM administration. Moreover, due to the lack of output data (C-T) in the publication by Cagnardi et al. (2010) (study C, D), only the key pharmacokinetic parameters were included in the comparative analysis, i.e. AUC_{0-t} – area under the

Table 2. Pharmacokinetic parameters and T>MIC_{90} of cefoperazone after single intramammary administration of the drug.

PK parameters	Unit	Cefoperazone M; (SD)			
		A	B	C	D
AUC_{0-t}	µg×h/L	6658871.33; (980039.08)	530930.40; (76881.15)	3279920.1; (574702.0)	13038885.2; (6849247.70)
AUC_{0-inf}	µg×h/L	6661106.98; (980155.01)	530975.04; (76878.08)	–	–
$AUMC_{0-t}$	µg×h2/L	33200895.11; (12369019.78)	7728350.40; (1032459.93)	32133172.9; (4418372.4)	39035135.6; (5461104.10)
$AUMC_{0-inf}$	µg×h^2/L	33433209.71; (12374293.06)	7733829.33; (1036110.24)	–	–
$AUC_{rest\%}$	%	0.03; (0.01)	0.01; (0.00)	–	–
MRT_{0-t}	h	4.85; (1.00)	14.58; (0.17)	10.33; (1.39)	3.82; (1.68)
k_{el}	1/h	0.051; (0.005)	0.038; (0.008)	–	–
k_d	1/h	0.502; (0.167)	0.155; (0.006)	–	–
B	mg/L	8.842; (3.452)	0.153; (0.113)	–	–
A	mg/L	6474.95; (4384.05)	251.46; (54.74)	–	–
$t_{1/2kel}$	h	13.70; (1.30)	19.29; (4.76)	5.18; (1.10)	5.16; (1.28)
$t_{1/2MRT}$	h	3.36; (0.69)	10.11; (0.12)	7.16; (0.96)	2.65; (1.16)
$t_{1/2kd}$	h	1.54; (0.50)	4.48; (0.17)	–	–
C_{max}	mg/L	1286.55; (154.76)	36.17; (5.77)	475.84; (73.64)	2450.88; (1040.69)
t_{max}	h	2.00; (0.00)	12.00; (0.00)	0.6; (0.1)	1.0; (0.7)
C_{last} t_{last}	µg/L h	28.11; (11.12) 84.00; (0.00)	2.20; (0.08) 108.00; (0.00)	– 72.0; (0.0)	 60.0; (0.0)
k_{10}	1/h	0.495; (0.168)	0.155; (0.005)	–	–
k_{21}	1/h	0.052; (0.005)	0.038; (0.008)	–	–
k_{12}	1/h	0.005; (0.002)	0.0002; (0.000)	–	–
k_{20}	1/h	5.098; (2.358)	39.99; (11.725)	–	–
CL	L/h	0.038; (0.005)	0.00048; (0.000)	0.114; (0.022)	0.039; (0.023)
V_1	L	0.057; (0.034)	0.0010; (0.000)	–	–
V_2	L	0.562; (0.333)	0.2948; (0.164)	–	–
T>MIC_{90}	h	50.47; (5.21)^{Ma}	40.21; (0.62)^{H}	45.0; (6.0)^{Ma}, 38.0; (5.0)^{H}	
T>$MIC_{90,DN}$	h	54.50; (5.86)^{Ma}	41.94; (0.60)^{H}		

A, B, C, D – studies described in Table 1; M – arithmetic mean; SD – standard deviation; AUC_{0-t} – area under the curve calculated between zero and last sampling point; AUC_{0-inf} – area under the curve calculated between zero and infinity; $AUMC_{0-t}$ – area under the first moment of curve calculated between zero and last sampling point; $AUMC_{0-inf}$ – area under the first moment of curve calculated between zero and infinity; MRT_{0-t} – mean residence time calculated for last sampling point; $AUC_{rest\%}$ – percent of the rest area under the curve; C_{max} – maximal concentration; t_{max} – time to reach maximal concentration; C_{last} – last measured concentration; t_{last} – time of last measured concentration; k_{el} – elimination rate constant; k_d – distribution rate constant; $t_{1/2kel}$ – elimination half-life; $t_{1/2MRT}$ – elimination half-life based on MRT_{0-t}; $t_{1/2kd}$ – distribution half-life; B – concentration extrapolated by elimination phase; A – concentration extrapolated by distributionąphase; k_{10} – elimination rate from milk compartment; k_{20} – elimination rate from tissue compartment; k_{12} – rate constant between milk and tissue concentration; k_{21} – rate constant between tissue and milk concentration; CL – total clearance; V_1 – milk compartment volume; V_2 – tissue compartment; T>MIC_{90} – time above MIC expressed for *S. aureus* by Cagnardi et al., (2012); T>$MIC_{90,DN}$ – dose normalized time above MIC_{90}; Ma – mastitis; H – healthy.

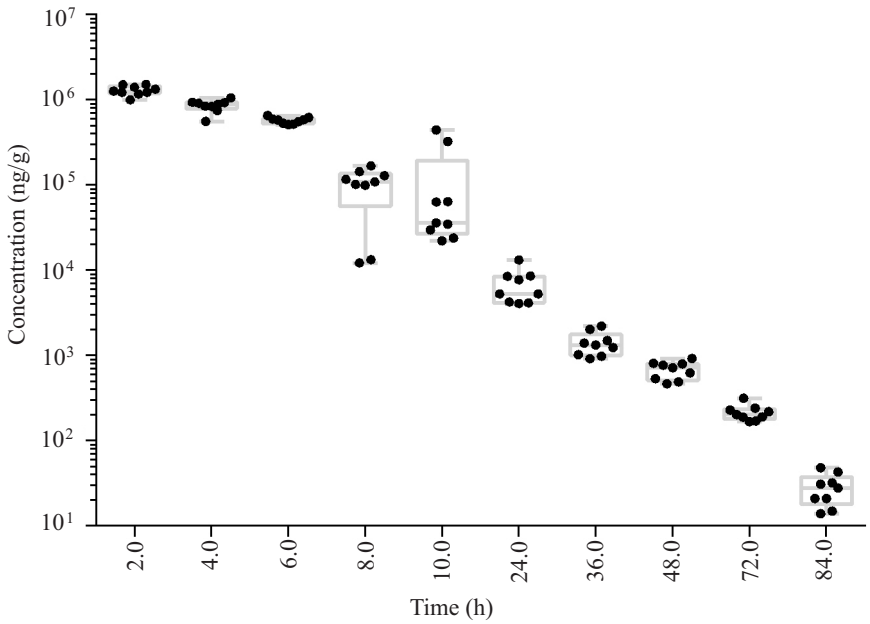

Fig. 1. Kinetics of cefoperazone withdrawal after single intramammary administration of the drug at a dose of 250 mg (n=9, study A).

curve calculated between zero and the last sampling point, $AUMC_{0\text{-}t}$ – area under the first moment curve calculated between zero and the last sampling point, $MRT_{0\text{-}t}$ – mean residence time calculated for the last sampling point, $t_{1/2kel}$ – elimination half-life, C_{max} – maximal concentration. In studies C and D, the clearance value was additionally calculated with the formula $CL = D/AUC_{0\text{-}t}$, where D denotes the drug dose administered to one quarter. The aforementioned parameters were also calculated in study A and B, including parameters $AUC_{0\text{-}inf}$ – area under the curve calculated between zero and infinity; $AUMC_{0\text{-}inf}$ – area under the first moment curve calculated between zero and infinity; $MRT_{0\text{-}inf}$ – mean residence time calculated for infinity; $AUC_{rest\%}$ – percent of the rest area under the curve; t_{max} – time to reaching maximal concentration; C_{last} – last measured concentration; t_{last} – time of the last measured concentration; k_{el} – elimination rate constant; k_d – distribution rate constant; $t_{1/2kd}$ – distribution half-life; B – concentration extrapolated by the elimination phase; A – concentration extrapolated by the distribution phase; k_{10} – elimination rate from the milk (central) compartment; k_{20} – elimination rate from the tissue compartment; k_{12} – rate constant between milk and tissue concentrations; k_{21} – rate constant between tissue and milk concentrations; V_1 – milk compartment volume; V_2 – tissue compartment.

In all studies (A, B, C, D), the $t_{1/2MRT}$ – elimination half-life based on $MRT_{0\text{-}t}$ was calculated using equation $t_{1/MRT} = Ln(2)/(1/MRT_{0\text{-}t})$.

The compartment analysis distinguished two compartments. The first one was the central compartment comprising milk present in the teat canal and teat sinuses. Udder tissues were defined as the second compartment. Therefore, the distribution rate constant k_d illustrates the elimination rate constant for drug withdrawal with milk. In turn, the elimination rate constant k_{el} represents drug transfer from milk to tissues.

Pharmacodynamic parameters analysis

In studies A and B, based on the minimal inhibitory concentration (MIC_{90}), the time during which the minimal inhibitory concentration was noted in 90% of the *S. aureus* population ($T>MIC_{90}$) was determined, likewise in the study conducted by Cagnardi et al. (2010), in which $T>MIC_{90}$ was determined from the concentration curve established on the basis of a dose of 300 mg/quarter. In turn, in studies A and B, a dose of 250 mg/quarter was used and the value normalised with the dose rate $T>MIC_{90,DN}$ was calculated. Hence, the raw data used for the pharmacokinetic calculations in studies A and B were multiplied × 1.2.

Statistical analysis

A t-Student test was employed to confirm the significance of these observations. Differences with a *p*-value <0.05 were considered significant.

Results

The results of the pharmacokinetics analysis of CEF in cow's milk after IMM administration are shown in Table 2 (study A, B, C, D) and Fig. 1 (study A). Recalculation of the PK parameters in studies B, C, and D revealed that AUC_{0-t} in study A was over 12-fold higher than that in study B, over 2-fold higher than that in study C, and by half lower than that calculated in study D. Simultaneously, it was found that $AUMC_{0-t}$ in study A was over 4-fold higher than that in study B, while the difference in $AUMC_{0-t}$ calculated in studies C and D was insignificant ($p>0.05$). As regards MRT_{0-t}, three different values were obtained in studies A, B, and C ($p<0.05$). The differences in the MRT_{0-t} values were not statistically significant ($p>0.05$) only in the case of study A and D. In studies A, B, and D, the ratio of $t_{1/2kel}$ to $t_{1/2MRT}$ was 4.07, 1.91, and 1.95, respectively. $t_{1/2MRT}$ was longer than $t_{1/2kel}$ only in study C.

For C_{max}, comparative analysis can only be carried out for studies A, C, and D due to the similar values of t_{max} in the range of 0.5-2h after administration of the drug. C_{max} in study D (t_{max} = 1h) was almost two-fold higher than C_{max} in study A (t_{max} = 1h) ($p<0.05$). In turn, C_{max} in study C (t_{max} = 0.6h) was 2.7-fold lower than that in study A (t_{max} = 1h) ($p<0.05$). Given the lack of analysis in the range of 0-12h after the administration of the drug, the CL value calculated in study B, which was strongly dependent on AUC_{0-t}, was not included in the comparative analysis. There was no significant difference between the CL values calculated in studies A and D. In turn, a significant difference was found between the CL values obtained in studies A, D, and C ($p<0.05$). In study C, the CL value was 3-fold higher than that in studies A and D. The time of complete CEF wash-out expressed as $10\times t_{1/2kel}$ in groups A, B, C, and D varied and amounted to 5.7, 8.0, 2.2, and 2.2 days after administration of the drug, respectively. In study A and B, in which it was possible to determine the compartmental model parameters, k_{el} k_{21} whereas k_d k_{10}.

The homogeneity of the results obtained was analysed. The RSD% was calculated for selected parameters. In the case of AUC_{0-t}, the RSD% in studies A, B, C, and D was 14.7, 14.5, 17.5, and 52.5%, respectively. As regards $AUMC_{0-t}$, the RSD% in studies A, B, C, and D was 37.3, 13.4, 13.8, and 14.0%, respectively. In the case of MRT_{0-t}, the RSD% was 20.7, 1.2, 13.5, and 43.9% in studies A, B, C, and D, respectively, while the $t_{1/2kel}$ value was 9.5, 24.7, 21.2, and 24.9%, respectively. In the case of C_{max} and CL, the RSD% in studies A, B, C, and D was 12.0, 16.0, 15.5, and 42.5 as well as 13.4, 14.7, 19.6, and 59.4, respectively.

The mean variability of AUC_{0-t}, $AUMC_{0-t}$, MRT_{0-t}, $t_{1/2kel}$, C_{max}, and CL in studies A, B, and C was at a similar level and amounted to 16.28%, whereas in the case of study D it differed from that reported in the other studies with a value amounting to 39.52%.

The results of the pharmacodynamic analysis comprising $T>MIC_{90}$ and $T>MIC_{90,DN}$ are presented in Table 2.

The proposed two-compartment model was verified by calculations. The k_d/k_{el} value was 9.83 and for 4.07 for study A and B, respectively. This implies that the processes referred to as distribution achieved 9- and 4-fold higher rates than the determined k_{el} in both cases.

Discussion

The failure to standardise the requirements for the level of CEF residues in milk by the Veterinary International Conference of Harmonisation still hampers consistent control of CEF residues on a global scale. In the EMA sphere of influence, the approved CEF residue level is 50 µg/kg, whereas no tolerance values have been determined by the FDA. In the US, IMM formulations containing ceftiofur and cephapirin are approved for use in dairy cattle. CEF in the IMM formulation has not been approved, and the extralabel use of cephalosporins in dairy cattle is prohibited (FDA 2012). One of the best and widely preferred methods for confirmatory analysis of residues includes chromatographic mass spectrometry techniques (Codex Alimentarius Commission 2016). In this study, the analyses were performed with the use of the HPLC-MS/MS technique and the results of the pharmacokinetic analysis were compared to those obtained in studies based on the microbiological method and HPLC-UV (Wilson et al. 1986, Cagnardi et al. 2010). The analytical method used in study A was 5-fold more sensitive than the methods used in studies B, C, and D. So far, no retrospective pharmacokinetic analysis of data obtained with the microbiological method after IMM administration of CEF has been performed. One of the findings of the comparison of the analysed investigations is that the CEF kinetics determined with the microbiological method 30 years ago (study B) is consistent with the results obtained by the authors of this paper with the use of the 5-fold more sensitive HPLC-MS/MS technique (study A). Studies A and B yielded similar results of the key pharmacokinetic parameters related to the level of the drug distribution to tissues (V_2) and $t_{1/2kel}$.

The scheduled t_{last} had a significant effect on proper determination of CEF $t_{1/2kel}$. In studies A-D, it ranged from 60 to 108 hours after administration of

the drug. The "gold standard" of sampling after single administration in pharmacokinetic analysis is taking samples at a period equal to 2-3 × $t_{1/2kel}$ after drug administration. However, this principle cannot be applied at IMM application, since the determination of the parameter is associated with the physiology of the alimentary tract rather than the mammary gland (Grabowski et al. 2012). If the arithmetic mean from studies A and B is assumed as the longest value CEF $t_{1/2kel}$ observed, the length of the investigations is significantly different in relation to $t_{1/2kel}$. Studies A, B, C, and D were conducted in a period of 5.0, 6.5, 4.4, and 3.6×$t_{1/2kel}$. Besides the differences in the methodology for determination of k_{el}, this is probably the main cause of such large discrepancies in the $t_{1/2kel}$ values between studies A, B and C, D. $AUC_{rest\%}$, whose value is a compilation of k_{el}, t_{last}, and LOQ, confirms the high quality of studies A and B. Unfortunately, calculation of this parameter for studies C and D was not possible. Assuming that the CEF wash-out equal to 90.90% proceeded in time equal to 10×$t_{1/2kel}$, depending on the analysed study (A, B, C, D), it ranged from 8.0 and 2.2 days after administration of the drug. As shown, many factors exert an effect on the $t_{1/2kel}$ value, which may yield significant differences in the description of the drug wash-out.

In studies A, B, and D, $t_{1/2MRT} < t_{1/2kel}$, which indicates that the analysed processes cannot be included in one-compartment kinetics. Studies A, B, and D relate to the two-compartment model of CEF distribution. In the case of study C, the drug elimination kinetics exhibited a course similar to the one-compartment model, since $t_{1/2MRT} > t_{1/2kel}$. The distribution of CEF to the tissue compartment is clearly distinguished with the kinetic phase. An important element in drug elimination is the CEF transfer from tissues to milk and transfer from udder tissues to the systemic circulation. However, V_2 is low, as confirmed by the analysis of data from study B. The rate constant k_{20}, illustrating the rate of elimination of CEF present in the systemic circulation after IMM administration, exhibits very high values (study A and B), which is consistent with the results obtained in studies C and D. Studies C and D revealed the presence of CEF in the systemic circulation after IMM drug administration. Analysis of data reported by Cagnardi's team indicates diverse values of $t_{1/2kel}$ for CEF present in blood after IMM administration, depending on the animal health status. Healthy (H) animals had $t_{1/2kel} \approx 7.00$ h and infected (I) individuals exhibited $t_{1/2kel} \approx 17.15$ h.

In all the three studies, milk was sampled for analyses at different time points and the investigations were completed at various times. The methods used differed in the sensitivity levels (LOQ). Different designs and assumptions of the analysed studies hamper the comparative analysis of the pharmacokinetic characteristics of CEF. The milk yields in studies A, C, and D were similar. In study B, however, the milk yield was at least 3-fold lower. Production of milk in the case of IMM application is one of the major elements modifying the kinetics of drug elimination. k_{10} is a rate constant with a value strictly correlated with the milk yield. In study A (milk yield of 25-30 L/24h) and B (milk yield of 10 L/24h), it was possible to determine the k_1 value, which was approximately 3-fold lower in study B compared with that in study A.

Assuming that k_{10} is the drug eliminated in milk, the rate of CEF elimination from tissues is 10- (study A) and 258-fold (study B) higher than the rate of drug wash-out with milk. The CEF transfer to tissue is a relatively slow process. This is indicated by the k_{21}/k_{12} ratio showing that the CEF transfer from milk to tissues in studies A and B is, respectively, 10- and 190-fold slower than that from tissues to milk. This fact also explains the very high rate of elimination from the tissue compartment (k_{20}).

In the pharmacodynamic analysis, the observations in all four studies were entirely consistent and confirmed the observations concerning different values of $T>MIC_{90}$ depending on the animal health status (H – healthy; Ma – *mastitis*) reported by Cagnardi. The $T>MIC_{90}$ values calculated in studies A, B, C, and D suggest that the milk yield has no significant effect on the value of this parameter in the case of CEF.

Conclusions

The retrospective data analysis confirmed the very high value of the data obtained with the microbiological method. The comparative analysis of studies in which the milk yield was high (A, C, D) and low (B) allows a conclusion that this parameter in the case of CEF has no significant effect on $T>MIC_{90}$ and $T>MIC_{90,DN}$. The comparative analysis of $T>MIC_{90}$ and $T>MIC_{90,DN}$ in studies A, B, C, and D has shown lower values of these parameters in healthy animals and significantly higher values in infected individuals. It was confirmed that not only the type of the analytical method but also correct sampling have a significant impact on determination of the correct value of the drug half-life. Hence, depending on the analysed study (A, B, C, D), the CEF wash-out rate of 90.90% ranges from 8.0 to 2.2 days after administration of the drug.

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