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Comparative pharmacokinetics of some veterinary specialties including enrofloxacin in dogs*)

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Summary

This study was aimed at the comparison of the pharmacokinetics of 4 different parenteral commercial specialties containing enrofloxacin as an active substance. For this purpose, 35 2-3-years-old, male mix-breed dogs were used. Five groups including 7 animals each were established. Each animal included in the first group (group 1) was intravenously administered a reference standard for enrofloxacin at a dose of 5 mg/kg body weight. Groups 2, 3, 4 and 5 were administered different parenteral preparations containing enrofloxacin at the same dose but intramuscularly. Subsequent to drug administration, blood samples were collected from all of the groups at 0.083, 0.25, 0.50, 1, 2, 4, 6, 12, 24, and 36 hours. The blood samples were centrifuged for the separation of sera. Enrofloxacin analyses in serum samples were performed by means of the spectrofluorometric method. Upon evaluation based on pharmacokinetic distribution and according to the findings obtained, a statistically significant difference was determined in other groups with respect to A_1, α, $t_{1/2\alpha}$, V_1 ve k_{10} when compared to administration by route IV. This difference was demonstrated to be in the form of a decrease for A_1*, α and k_{10}, and increase for $t_{1/2\alpha}$ and V_1, when compared to administration by route IV. Excluding $t_{1/2\alpha}$, statistically significant differences were not observed between all the commercial preparations (groups 2-5).

Keywords: veterinary, commercial specialties, enrofloxacin, dogs

Enrofloxacin is an antibacterial compound that has been selected for veterinary use only and which is a quinolone carboxylic acid derivative (1). It is a broad spectrum antibiotic with efficacy against mostly aerobic bacteria, as well as facultative anaerobic bacteria, *Mycoplasma spp.* and *Rickettsia spp.* The presence of a fluoride group in the compound increases efficacy against Gram (positive) and aerobic bacteria (9, 14). Chromosomal changes constitute the main mechanism of resistance to fluoroquinolones, and changes in the target enzyme (DNA girase) result in a decrease in the passage of fluoroquinolones into bacteria (8, 17, 18, 24). Enrofloxacin is efficacious against Gram (negative) bacteria and *Mycoplasma* spp. as well as Gram (positive) bacteria at very low concentrations (2, 3).

The administration of enrofloxacin in association with antacids containing magnesium and aluminium decreases the bioavailability of the antibiotic (10, 22). In case the drug is taken with food, peak plasma concentrations differ to a small extent; however, the area under the concentration curve ($AUC_{0\rightarrow\infty}$) or the excretion-half life does not change (12). One of the most striking pharmacokinetic characteristics of fluoroquinolone antibiotics is their broad volume of distribution (1.5-3 L/kg). Moreover, their level of binding to plasma proteins is lower than 50% (12, 19). Enrofloxacin passes into all body compartments and is found in high concentrations particularly in bile, the liver, the lungs and the kidneys. Its concentration in the lungs may be many times greater than its plasma concentration (9).

The present study aimed at the comparison of the pharmacokinetics of 4 specialties containing 10% enrofloxacin, placed on the market for veterinary use, appropriate for parenteral use and presented in flacons.

Material and methods

Animals. 35 two-three-year-old male dogs of approximately the same body weight were used in this study. Each animal was weighed in order to establish full homogeneity with respect to live weight, and 5 groups, each including 7 animals, were established. The protocol of this study was approved by Ethics Committee, Faculty of Veterinary Medicine, University of Erciyes.

Application. In the first group (group 1), the active substance of enrofloxacin was mixed in a buffer solution with

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a pH value close to that of the body, so that it could readily dissolve, and was administered to each animal at a dose of 5 mg/kg body weight intravenously. Groups 2, 3, 4 and 5 were administered different parenteral preparations containing enrofloxacin as an active substance, at the same dose, but intramuscularly route. All specialties were produced by drug companies in Turkey.

Collection of blood samples. Following the administration of the specialties, blood was collected from all groups, into tubes not containing anticoagulants, at 0.083, 0.25, 0.50, 1, 2, 4, 6, 12, 24, 36 hours. Serum was obtained from the samples by means of centrifugation at 3000 rpm. Sera were transferred into eppendorf tubes and stored at –20°C until analyzed.

Extraction of samples. Serum extraction and the preparation of extracted sera for measurements were performed in accordance with the method described by Rizk et al. (15) with minor modifications. Accordingly, 7 ml of methanol was placed into a 10 ml-dry tube and 1 ml of serum was added using an automatic pipette. The cap of the tube was closed and the tube was vortexed for 1 minute. Subsequently, the tube was centrifuged at 4500 rpm for 10 minutes. The supernatant was transferred into a separate tube by filtering through a Watman 42 filter paper.

Preparation of devices for measurement and analysis of measurements. Two ml of acetate buffer (pH 5) was added to the extracted sample, the mixture was vortexed and distributed into quartz baths. The spectrum of enrofloxacin was drawn in the emission mode of the device, using the RFPC programme, at an excitation wave length of 277 nm and between 240-600 nm. Subsequently, absorbances were calculated at 420-520 nm by using the same programme.

Drawing of the standard curve and calculation of the serum antibiotic concentrations. For the drawing of the standard curve, firstly, an enrofloxacin stock 1 solution of a concentration of 10 mg/100 ml was prepared in methanol. On the other hand, similar to the analyses of samples, the same procedures were repeated for the serum not containing any antibiotics, and 1 ml of canine serum free from antibiotics and 7 ml of methanol was placed into 10 ml--tubes. The mixture was first vortexed for 1 minute and then centrifuged at 4500 rpm for 10 minutes. The supernatant was filtered and collected into a separate balloon gauge. Subsequently, 2 ml of acetate buffer (pH 5) was measured for each tube and added to the balloon into which methanol had been filtered. Thereby all the procedures applied during the extraction of samples were also applied in the same order for the serum not containing antibiotics. The stock 2 solution of enrofloxacin was prepared from its stock 1 solution, and diluted at certain rates so as to be used for the drawing of the standard curve of enrofloxacin.

Recovery procedures. Prior to the start of the trial, blood samples collected from the animals into dry tubes were centrifuged at 3000 rpm for 10 minutes for the separation of sera. Sera were extracted by means of the addition of a certain amount of enrofloxacin stock solution dissolved in methanol, and subsequently measurements were performed. The same extraction procedure was performed beforehand by using blind serum, the extraction fluid was added in the same amount, and measurements were performed.

Antibiotic analyses. Measurements were performed by means of a spectrofluorophotometer device: the spectra of samples ready for measurement were transferred to a computer by means of a RFPC program in the spectrofluorophotometer device (Shimadzu RF-5301 PC). Subsequently, calculations were made in µg/ml by means of the same program, based on absorbance in certain regions. The calculated concentrations were multiplied with a dilution coefficient and serum enrofloxacin levels were determined.

Pharmacokinetic calculations. In the case of intravenous administration, the distribution model of the drug was determined according to r^2 values assessed upon the regression analysis of the drug plasma concentration-time curve. Subsequently, pharmacokinetic calculations (A_1*, A_2*, A_3* mathematical coefficients; k_a, first order absorption rate constant; α, hybrid rate constant for distribution phase; β, hybrid rate constant for terminal elimination phase; $t_{1/2a}$, absorption half life; $t_{1/2\alpha}$, half life at α phase; $t_{1/2\beta}$, half life at β phase; MRT, mean residence time; $AUC_{0\rightarrow\infty}$, area under the concentration time curve; V_1, volume of distribution in central compartment; k_{12}, distribution rate constant for transferring the drug from the central to peripheral compartment; k_{10}, elimination rate constant; k_{21}, transfer from the peripheral to central compartment; V_{dss}, volume of distribution at steady state; Cl_{total}, total plasma clearance; C_{max}, maximal concentration in plasma after intra muscular administration; t_{max}, time needed to reach C_{max}; A_2/A_1, central compartment drug amount/peripheral compartment drug amount; F, bioavailability) were made in accordance with the distribution model of the drug. Calculations were made by means of the PKCALC and CWBASIC program including the equations reported by Shumaker (16) and Wagner (23).

Statistical calculations. The SPSS 10.0 statistical software package for Windows was used for statistical calculations. Data was given in the form of arithmetical mean values and ± standard deviations. Differences between groups were evaluated according to the one-way analysis of variance (ANOVA). Different groups were determined by means of the Duncan test.

Results and discussion

Numerous studies have been carried out on the pharmacokinetics of enrofloxacin in dogs (3, 4, 7, 11, 13) and other animals (5, 6, 20, 21). No previous study exists on the comparison of specialties manufactured in other countries and our countries in dogs.

The character of the standard curves of enrofloxacin drawn by means of the determination of absorbance at 10 nm intervals between the 420-520 ranges, based on the excitation spectrum drawn under the conditions indicated above, was demonstrated to be linear. The linearity was determined to continue at intervals of 0.025-2.000 µg/ml, and the r^2 value was observed to fall within the interval of 0.951-0.987 for curves drawn at a total of ten points. The limit of detection of the analytic method for enrofloxacin was 0.025 µg/ml, and the quantition of detection of serum was 0.18 µg/ml. The value determined in recovery studies was 98.12%.

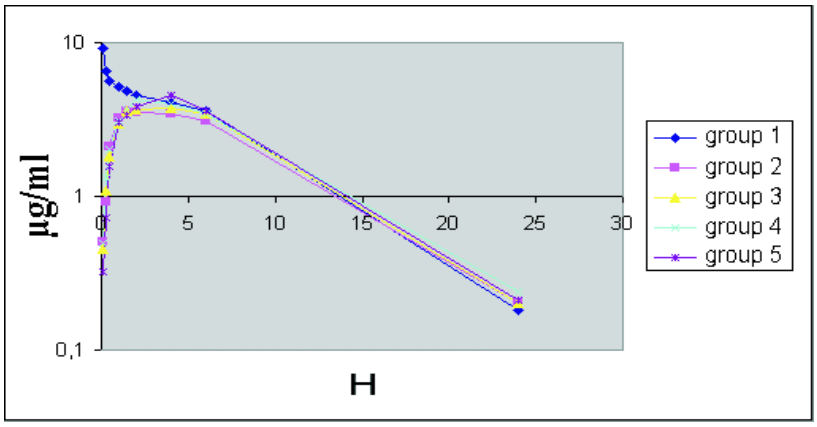

Fig. 1. Serum concentration-time profile following enrofloxacin standard intravenous administration (group 1) and intramuscular administration of four different commercial preparations (groups 2 and 5)

According to the evaluation of the drug serum concentration-time curve, drawn on the basis of the analysis results of the blood samples collected at certain periods subsequent to the administration of the drug, and in light of the results of the regression analyses, the distribution of enrofloxacin in dogs was found to be more consistent with the open two compartmental model (fig. 1).

Subsequent to the administration of the active substance intravenously, serum drug concentrations at 0.083, 0.25, 1, 6 and 24 hours were determined to be 9.18 µg/ml, 6.56 µg/ml, 5.20 µg/ml, 3.60 µg/ml and 0.18 µg/ml, respectively. The level of the probable existent drug in the serum by the end of the 36th hour was demonstrated to be below detectable limits in the collected blood samples. Subsequent to the administration of the drug by intramuscular route, serum drug concentrations at 0.083, 0.25, 1, 2, 4 and 24 hours in group 2, group 3, group 4 and group 5 were determined as 0.50, 1.32, 2.12, 3.22, 3.46, 0.20 µg/ml; 0.22, 1.42, 1.83, 2.28, 2.59, 0.20 µg/ml; 0.50, 1.47, 2.01, 3.09, 3.97, 0.24 µg/ml and 0.32, 1.55, 3.00, 3.82, 4.50, 0.21 µg/ml, respectively (fig. 1). According to the obtained findings, a statistically significant difference amongst the evaluated parameters was determined only with respect to A_1, α, $t_{1/2\alpha}$, V_1 and k_{10} between intravenous administration and the other groups ($p < 0.05$). In the case of administration by the intramuscular route, statistically significant differences did not exist except for $t_{1/2\alpha}$ ($p > 0.05$) (tab. 1).

In conclusion, as a result of examinations performed in veterinary commercial formulations containing 10% enrofloxacin, presented in the form of flacons, and for parenteral use, no statistically significant difference existed between the specialties except for $t_{1/2\alpha}$.

Tab. 1. Some pharmacokinetic parameters of enrofloxacin after i.v. administrations of drug standard (group 1) and i.m. administrations of four commercial formulations (groups 2-5) in dogs as a single dose of 5.0 mg/kg/b.w.

Parameters**	Group 1	Group 2 (Reference)	Group 3 (Test 1)	Group 4 (Test 2)	Group 5 (Test 3)
A_1* (µg/ml)	14.36 ± 11.87[a]	−5.12 ± 5.72[b]	−6.98 ± 3.65[b]	−6.96 ± 6.08[b]	−6.55 ± 2.12[b]
A_2* (µg/ml)	6.55 ± 0.61	7.60 ± 4.78	9.10 ± 3.72	9.84 ± 5.30	8.62 ± 1.31
A_3* (µg/ml)	–	−4.25 ± 2.30	−3.00 ± 2.30	−3.18 ± 1.79	−3.26 ± 2.79
k_a (h^{-1})	–	4.66 ± 4.73	5.81 ± 6.90	1.37 ± 1.15	4.74 ± 6.32
α (h^{-1})	17.90 ± 7.42[a]	0.44 ± 0.13[b]	0.72 ± 0.24[b]	0.33 ± 0.15[b]	0.44 ± 0.12[b]
β (h^{-1})	0.14 ± 0.07	0.13 ± 0.03	0.99 ± 1.92	0.69 ± 1.06	0.15 ± 0.02
$t_{1/2a}$ (h)	–	0.34 ± 0.31	0.28 ± 0.23	0.52 ± 0.31	0.32 ± 0.20
$t_{1/2\alpha}$ (h)	0.04 ± 0.01[a]	1.66 ± 0.51[c]	1.33 ± 0.21[b]	1.75 ± 0.60[c]	1.64 ± 0.35[c]
$t_{1/2\beta}$ (h)	4.59 ± 0.22	5.32 ± 1.31	5.60 ± 2.51	4.92 ± 1.26	4.64 ± 0.54
MRT (h)	6.72 ± 0.38	8.73 ± 1.09	9.33 ± 3.23	8.66 ± 1.64	8.25 ± 1.29
$AUC_{0\to\infty}$ (mg/h/L)	62.12 ± 7.14	59.96 ± 24.72	61.43 ± 13.24	52.84 ± 22.17	59.00 ± 11.80
V_1 (L/kg)	0.28 ± 0.09[a]	1.85 ± 0.92[b]	1.63 ± 0.63[b]	1.22 ± 0.52[ab]	2.10 ± 1.41[b]
k_{12} (h^{-1})	11.56 ± 7.30	–	–	–	–
k_{10} (h^{-1})	0.45 ± 0.27[a]	0.08 ± 0.04[b]	0.06 ± 0.02[b]	0.09 ± 0.03[b]	0.07 ± 0.03[b]
k_{21} (h^{-1})	–	0.97 ± 0.56	1.39 ± 0.55	0.79 ± 0.55	1.11 ± 0.54
V_{dss} (L/kg)	4.51 ± 0.91	–	–	–	–
Cl_{total} (L/h/kg)	0.11 ± 0.01	0.13 ± 0.04	0.09 ± 0.01	0.10 ± 0.03	0.12 ± 0.01
C_{max} (µg/ml)	–	4.18 ± 0.68	4.20 ± 0.79	4.36 ± 1.38	4.70 ± 1.49
t_{max} (h)	–	2.94 ± 1.31	3.57 ± 1.96	2.42 ± 1.61	3.42 ± 0.97
A2/A1	0.67 ± 0.28	–	–	–	–
F (%)		96.52	98.88	85.06	94.97

Explanations: a, b, c – Means within the same line with different letters are statistically significant ($P < 0.05$). ** – A_1*, A_2*, A_3* mathematical coefficients; k_a – first order absorption rate constant; α – hybrid rate constant for distribution phase; β – hybrid rate constant for terminal elimination phase; $t_{1/2a}$ – absorption half life; $t_{1/2\alpha}$ – half life at α phase; $t_{1/2\beta}$ – half life at β phase; MRT – mean residence time; $AUC_{0\to\infty}$ – area under the concentration time curve; V_1 – volume of distribution in central compartment; k_{12} – distribution rate constant for transferring the drug from the central to the peripheral compartment; k_{10} – elimination rate constant; k_{21} – transfer from peripheral to central compartment; V_{dss} – volume of distribution at steady state; Cl_{total} – total plasma clirens; C_{max} – maximal concentration in plasma after intra muscular administration; t_{max} – time needed to reach C_{max}; A_2/A_1 – central compartment drug amount/peripheral compartment drug amount; F – bioavailability

Tab. 2. Natural logarithms of bioequivalance parameters of enrofloxacin reference and test formulations in dogs

Preparations	Bioequivalance Parameters*				
	C_{max}	t_{max}	MRT	$t_{1/2ß}$	$AUC_{0\to\infty}$
Reference	0.62	0.46	0.94	0.72	1.77
Test 1	0.62	0.55	0.96	0.74	1.78
Test 2	0.63	0.38	0.93	0.69	1.72
Test 3	0.67	0.53	0.91	0.66	1.77
μ_{T1}/μ_R	1.00	1.19	1.02	1.02	1.00
μ_{T2}/μ_R	1.01	0.82	0.98	0.95	0.97
μ_{T3}/μ_R	1.08	1.15	0.96	0.91	1.00

Explanations: * – there was no statistically significant difference of in between pharmacokinetic parameters of natural logarithms of reference and test formulations ($P > 0.05$). μ_T/μ_R – represent the ratio of values of some pharmacokinetic parameters for reference and test formulations. For the equivalence of all three test formulations to the reference preparation parameters indicated above should be in between 0.80-1.25

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