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Comparative pharmacokinetics of various sulfadoxine-trimethoprim preparations used on dogs in veterinary medicine*)

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Summary

Twenty-eight mix-breed male dogs of approximately 3 years of age and with similar body weights were used in the study. Four groups, each including 7 animals, were established. The animals included in the first, second, third, and fourth groups were administered formulations A, B, C, and D, respectively, containing 200 mg sulfadoxine and 40 mg trimethoprim per millilitre, at a dose of 25 mg/kg body weight sulfadoxine by intramuscular route at the same site of the body. Subsequently, blood samples were collected at 0.083, 0.25, 0.50, 1.0, 1.5, 2.0, 4.0, 6.0, 12 and 24 hours. The levels of sulfadoxine-trimethoprim in the plasma samples were measured by means of a HPLC device. Pharmacokinetic calculations were performed in compliance with the two-compartment open model. According to statistical analyses, amongst the parameters evaluated, significant differences were determined to exist between the groups only with respect to the mathematical coefficients (A_2*), hybrid rate constant for terminal elimination phase (β), half life at α phase ($t_{1/2\alpha}$), half life at β phase ($t_{1/2\beta}$) and mean residence time (MRT) values for sulfadoxine, and with respect to the A_1*, first order absorption rate constant (k_a), β and MRT values for trimethoprim ($p < 0.05$).

Keywords: comparative pharmacokinetics, sulfadoxine-trimethoprim, dog

The first drugs to be used for the treatment and prevention of bacterial infections were sulphonamides. Today, despite a wide variety of specific drugs available for use in bacterial and parasitic infections, sulphonamides are still used extensively due to their broad spectrum, low toxicity and ease of administration and dosage, as well as for economic reasons. Sulfadoxine-trimethoprim formulations create a synergistic interaction through the inhibition of the enzymes involved in the synthesis reaction of folic acid in bacteria and coccidia (dihydropteroat synthetase and dihydrofolate reductase). Furthermore, although these drugs generally exhibit bacteriostatic effect when administered alone, they cause bacteriocide effect upon administration in the form of a dual combination. The sulfadoxine-trimethoprim combination has quite a broad spectrum, mainly against *streptococci*, *staphylococci*, *Nocardia spp.*, *enterobacteria* and certain protozoa (*coccidia*, *pneumocysts*, *toxoplasma*). This combination is used successfully in the treatment of the diseases of the respiratory, digestive, urinary and genital systems, and soft tissues in many animals. Generally, the sulphonamide portion is taken into account for the calculation of the dose of sulphonamide-trimethoprim combinations to be used (4, 6, 8-10, 12).

In the present study, the comparative pharmacokinetics of commercial formulations containing sulfadoxine-trimethoprim, placed on the market under different trade names, were investigated in dogs.

Material and methods

Twenty-eight male mix-breed dogs of approximately 3 years of age and with similar body weights, which were determined to be healthy upon examinations performed at the Department of Internal Medicine of the Faculty of Veterinary Medicine were used. In order to ensure exact homogeneity with respect to body weight within the groups, the animals were weighed separately, and four groups, each including 7 animals, were established. Animals were included randomly in the test groups. The animals included

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in the first, second, third, and fourth groups were administered formulations A, B, C, and D, respectively, containing 200 mg sulfadoxine and 40 mg trimethoprim per millilitre, at a dose of 25 mg/kg body weight sulfadoxine by intramuscular route at the same site of the body. Following administration, blood samples were collected from all of the groups into heparinised tubes at 0.083, 0.25, 0.50, 1.0, 1.5, 2.0, 4.0, 6.0, 12 and 24 hours. The blood samples were centrifuged for the separation of plasma.

The extraction of the plasma samples was carried out in compliance with the method described by Ascalone (1). The analysis of sulfadoxine and trimethoprim in plasma was performed using HPLC according to minor modifications of Fuerte et al.'s (2) and Ascalone's (1) methods. The curve of linearity, coefficient of variation for the repeated measurement, limit of detection (LOD), limit of quantification (LOQ) and recoveries of the plasma samples were calculated. Pharmacokinetic calculations were made by means of the PKCALC programme including equations by Shumaker (11). Statistical analyses were performed using the „SPSS 11.0 for Windows" statistical software package. Data was given in arithmetic means and ± standard deviations. The one-way analysis of variance (ANOVA) was used for the evaluation of differences between groups ($p < 0.05$). Different groups were determined using Duncan's test.

Tab. 1. Pharmacokinetic parameters of some preparations in intramuscular application for sulfadoxine in dogs

Parameters	Group 1	Group 2	Group 3	Group 4
A_1* (µg/ml)	21.94 ± 14.97	22.40 ± 20.57	2.66 ± 18.87	12.87 ± 4.13
A_2* (µg/ml)	33.66 ± 5.48^{b}	21.55 ± 4.01^{d}	38.72 ± 15.45^{a}	25.74 ± 6.21^{bc}
A_3* (µg/ml)	–59.57 ± 18.64	–44.64 ± 20.29	–32.27 ± 22.42	–41.00 ± 13.10
k_a (h^{-1})	1.88 ± 0.35	2.44 ± 2.40	1.38 ± 1.28	1.11 ± 0.60
α (h^{-1})	0.48 ± 0.30	0.56 ± 0.16	0.36 ± 0.19	0.33 ± 0.18
β (h^{-1})	0.03 ± 0.00^{ab}	0.01 ± 0.00^{c}	0.04 ± 0.01^{a}	0.02 ± 0.01^{bc}
$t_{1/2a}$ (h)	0.31 ± 0.15	0.42 ± 0.19	0.73 ± 0.34	0.74 ± 0.27
$t_{1/2\alpha}$ (h)	2.06 ± 1.32^{a}	1.34 ± 0.44^{a}	2.38 ± 1.13^{b}	3.01 ± 2.18^{b}
$t_{1/2\beta}$ (h)	20.63 ± 2.60^{ab}	40.53 ± 8.32^{c}	16.46 ± 7.05^{a}	28.54 ± 9.22^{b}
MRT (h)	29.60 ± 4.17^{a}	58.15 ± 10.98^{c}	24.52 ± 9.76^{a}	41.04 ± 13.10^{b}
$AUCt_{0\to24}$ (µg.h/ml)	547.75 ± 78.84	434.66 ± 47.96	536.44 ± 99.59	461.57 ± 137.18
C_{max} (µg/ml)	39.73 ± 10.52	35.02 ± 10.09	37.73 ± 8.12	33.84 ± 9.58
t_{max} (h)	2.21 ± 0.80	2.07 ± 0.88	2.14 ± 0.85	1.85 ± 0.24

Explanations: a, b, c, d – 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; $AUCt_{0\to24}$ – area under the concentration-time curve; C_{max} – maximal concentration in plasma after intramuscular administration; t_{max} – time needed to reach C_{max}

Results and discussion

Various studies have been performed on the pharmacokinetics of sulfadoxine and trimethoprim on some animals (3, 5, 7, 13, 14). No previous study exists on the use of these drugs on dogs.

The recovery of sulfadoxine and trimethoprim were determined as approximately 90.10% and 91.32% respectively. Coefficient of variation for the repeated inter-day measurements, LOD, and LOQ were calculated as approximately 2-4%, 3-6% for 3 different concentrations; 0.06 µg/ml, 0.03 µg/ml and 0.17 µg/ml, 0.10 µg/ml for sulfadoxine and trimethoprim, respectively. Upon the evaluation of r^2 values in the light of the results of regression analyses, the curve drawn was determined to display a linear character and to be 0.999 for each of the two drugs.

Based on the evaluation of the drug plasma concentration-time curve drawn according to the results of analyses performed in blood samples taken at certain intervals following the administration of the drug by intramuscular route, and the results of regression analyses, sulfadoxine and trimethoprim were determined to be more compatible with the two-compartment open disposition model. Pharmacokinetic calculations were

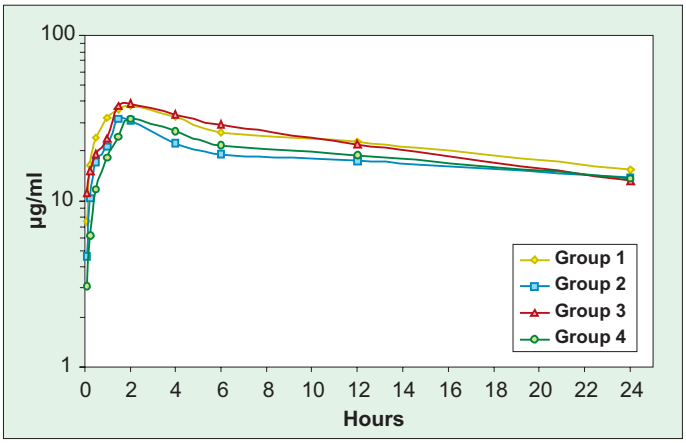

Fig. 1. Plasma concentration-time curve of some preparations in intramuscular application for sulfadoxine in dogs

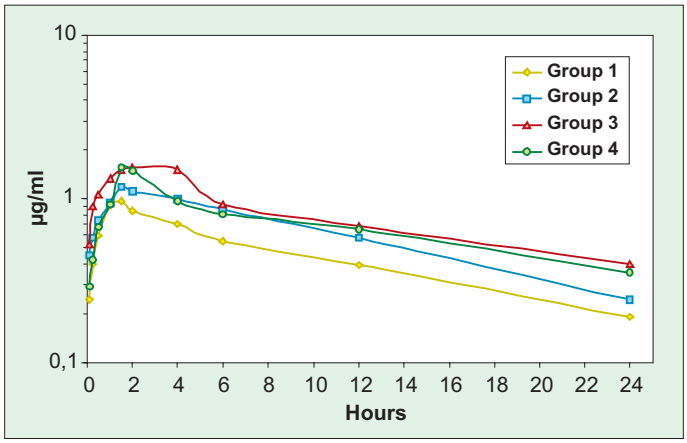

Fig. 2. Plasma concentration-time curve of some preparations in intramuscular application for trimethoprim in dogs

Tab. 2. Pharmacokinetic parameters of some preparations in intramuscular application for trimethoprim in dogs

Parameters	Group 1	Group 2	Group 3	Group 4
A_1* (µg/ml)	0.41 ± 0.35^{ab}	−0.38 ± 1.15^{a}	1.32 ± 0.53^{a}	1.53 ± 1.36^{b}
A_2* (µg/ml)	0.81 ± 0.49	1.32 ± 0.53	1.34 ± 0.72	1.05 ± 0.42
A_3* (µg/ml)	−1.18 ± 0.26	−0.98 ± 0.42	−1.45 ± 0.85	−2.09 ± 1.39
k_a (h^{-1})	1.91 ± 0.60^{a}	1.30 ± 1.19^{b}	1.76 ± 0.40^{ab}	1.26 ± 0.33^{b}
α (h^{-1})	0.49 ± 0.20	0.51 ± 0.29	0.32 ± 0.12	0.53 ± 0.25
β (h^{-1})	0.05 ± 0.02^{a}	0.05 ± 0.03^{a}	0.02 ± 0.02^{b}	0.03 ± 0.01^{ab}
$t_{1/2a}$ (h)	0.39 ± 0.12	0.47 ± 0.17	0.35 ± 0.17	0.57 ± 0.12
$t_{1/2\alpha}$ (h)	1.78 ± 1.20	2.11 ± 1.80	2.12 ± 1.06	1.42 ± 0.84
$t_{1/2\beta}$ (h)	11.80 ± 3.33	10.14 ± 1.30	12.49 ± 1.58	15.51 ± 2.62
MRT (h)	16.55 ± 4.40^{a}	15.20 ± 1.78^{a}	17.60 ± 1.76^{a}	22.35 ± 2.92^{b}
$AUCt_{0\rightarrow24}$ (µg.h/ml)	8.62 ± 6.24	13.49 ± 4.39	19.38 ± 12.52	15.03 ± 6.67
C_{max} (µg/ml)	1.04 ± 0.25	1.30 ± 0.44	1.70 ± 0.79	1.75 ± 0.46
t_{max} (h)	1.42 ± 0.34	1.92 ± 0.93	2.28 ± 1.21	1.71 ± 0.26

Explanations: a, b – means within the same line with different letters are statistically significant ($p < 0.05$)

based on this model. The mean plasma drug concentration levels of the commercial preparations (groups 1-4) according to the drug plasma concentration-time curve drawn at 0.083, 0.25, 0.50, 1.0, 1.5, 2.0, 4.0, 6.0, 12 and 24 hours, following the administration of the drug by intramuscular route, were shown in fig. 1-2. Based on the findings obtained, amongst the parameters evaluated, statistically significant differences between groups were determined to exist only with respect to the mathematical coefficients (A_2), hybrid rate constant for terminal elimination phase (β), half life at α phase ($t_{1/2\alpha}$), half life at β phase ($t_{1/2\beta}$) and mean residence time (MRT) values for sulfadoxine, and with respect to the A_1, first order absorption rate constant (k_a), β and MRT values for trimethoprim ($p < 0.05$) (tab. 1-2).

In conclusion, four preparations for parenteral use, available on the market and containing the active substance of sulfadoxine-trimethoprim, were studied with respect to certain pharmacokinetic parameters. In result, significant differences were determined between the four preparations for only some pharmacokinetic parameters.

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