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STUDY ON THE PHARMACOKINETICS OF TOBRAMYCIN IN DOGS

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Summary

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The pharmacokinetics of 4% tobramycin sulfate solution following a single i.v. application to six adult dogs (3 from each gender) at the dose of 3 mg/kg was investigated. The blood plasma antibiotic levels were determined using *Bacillus subtilis* ATCC 6633 as a test microorganism. The limit of quantification was 0.010 µg/mL. Plasma tobramycin pharmacokinetics was analysed via the compartmental and non-compartmental methods using a computer program (Topfit v. 2.0). The pharmacokinetic model was chosen by means of the Akaike's information criterion (AIC).

The determination of pharmacokinetic parameters of tobramycin in aqueous humour was done by the non-compartmental pharmacokinetic method.

After the single intravenous injection of tobramycin sulfate, its plasma biological half-life calculated according to the non-compartmental and the compartmental methods was 1.30±0.20 h and 0.87±0.09 h respectively. The total body clearance was 0.97±0.08 mL/min/kg and 0.91±0.20 mL/min/kg and the area under the concentration-time curve – 54.05±4.68 and 57.59±5.32 µg.h/mL respectively. The steady-state volume of distribution was 0.06±0.01 L/kg (via both methods).

Peak tobramycin concentrations in aqueous humour (2.24±0.29 µg/mL) were attained by hour 2 after the intravenous application. The biological half-life of tobramycin in aqueous humour was 3.04±0.21 h. The ratio of $AUC_{0\rightarrow\infty}$ values of aqueous humour and those of blood plasma, was 0.25.

Key words: aqueous humour, dogs, pharmacokinetics, tobramycin

INTRODUCTION

The proper antibacterial therapy is not possible without taking into consideration the sensitivity of a given pathogenic agent to the used chemotherapeutical drug, the determination of its concentration at the site of the lesion and its pharmacokinetics. All those factors influence the outcome of the etiotropic therapy.

The determination of antibacterial drug concentrations in aqueous humour and their pharmacokinetics following a systemic application is especially important for the efficient treatment of ophthalmic bacterial infections. Despite the reported data about tobramycin pharmacokinetics in different animal species (Regamey *et al.*,1973; Péchere *et al.*, 1976; Blouin *et al.*, 1979; Ling *et al.*, 1981; Jernigan *et al.*, 1988; Brown & Riviere, 1991; Hadi *et al.*, 1994; Christinsen *et al.*, 1996; Dimitrova *et al.*, 1998; Lashev and Dimitrova, 2002), humans (Regamey *et* *al.*, 1973; Blouin *et al.*, 1979; Counts *et al.*, 1982; Durmaz *et al.*, 1997) there is no information for plasma and aqueous humour tobramycin levels and its pharmacokinetics in dogs.

The aim of the present study was to determine plasma and aqueous humour tobramycin concentrations as well as its pharmacokinetics after intravenous administration to dogs.

MATERIALS AND METHODS

Animals

The study was performed on 6 clinicaly healthy mixed-breed dogs, aged 2-5 years, weighing 18-26 kg. They were housed in individual metal cages with dimensions corresponding to canine hygienic requirements, at ambient temperature of 17-19 °C, humidity of 58–60 % and 12 h light/12 h dark period light regimen. During a 20-days period of accomodation, the animals were fed twice daily with dry commercial food and given water *ad libitum*. A day prior to the experiment, the dogs underwent a complete physical examination, haematological (haematocrit, haemoglobin, erythrocyte counts, total and differential leukocyte counts) and blood biochemical analysis (ASAT, ALAT, creatinine, blood glucose, urea and alkaline phosphatase).

Experimental design

After a 12-hour fasting, the dogs were administered a single intravenous bolus dose of 3 mg/kg tobramycin sulfate (Tobramycin 4 % injectable solution, Balkanpharma, Razgrad, Bulgaria) in the right *v. cephalica*.

Blood samples of 1.5 mL were obtained by a "Venflon" cannula (Vygon, G-22, L-25 mm, ∅ 0.9 mm, Germany) from the left *v. cephalica* immediately prior to the treatment and 0.08, 0.25, 0.50, 1, 2, 4, 8 and 24 h after in heparinized Eppendorf tubes. Blood samples were centrifuged for 10 min at 3500 rpm, blood plasma was collected and divided in 2 aliquots of 300 µL in separate tubes.

Aqueous humour (300 µL) was obtained following a preliminary local anaesthesia of the eye with 2 % lidocaine solution by 0.5 mL disposable sterile syringes (Sherwood Medical, Northern Ireland) and G-28 injection needles (L-13 mm, ∅ –0.36 mm) from the anterior ocular camera. Obtained samples were stored at –18 °C for 18 h.

Determination of plasma and aqueous humour tobramycin concentrations

Plasma and aqueous humour concentrations of tobramycin were microbiologically determined by agar gel diffusion with *Bacillus subtilis* ATCC 6633 as a test organism. The standard solutions were prepared with tobramycin sulfate (biological activity of 948 µg/mg). The limit of quantification of the method was 0.010 µg/mL, the linearity (r^2) –0.986, and the percentage of recovery –93.64 %.

Pharmacokinetic analysis

Plasma and aqueous humour tobramycin concentrations were analysed by the TopFit v. 2.0 computer programme (Heinzel, *et al.*, 1993). The pharmacokinetic parameters of plasma tobramycin were determined using the compartmental (Baggot, 1977) and the non-compartmental pharmacokinetic analysis (Gibaldi & Perrier, 1982). The optimal pharmacokinetic model was selected on the basis of the Akaike's information criterion (AIC) (Yamaoka *et al.*, 1978).

Aqueous humour antibiotic levels were determined by the non-compartmental pharmacokinetic analysis (Gibaldi and Perrier, 1982).

RESULTS

The physical examination of dogs as well as the haematological and biochemical laboratory investigations revealed no deviation from the normal values. The average plasma and aqueous humour antibiotic concentrations are shown on Fig. 1. Plasma antibiotic levels were present by post administration min 5 (0.08 h) – 80.18±9.07 µg/mL, and remained up to hour 8 over 0.34 µg/mL. By hour 24 after the intravenous injection, tobramycin concentrations were under the sensitivity limit of the used method, i.e. below 0.010 µg/mL. The decline of tobramycin levels was characterized by a distribution phase up to hour 4 with $t_{1/2\alpha}$ – 0.07±0.02 h, followed by a slower elimination phase ($t_{1/2\beta}$ – 0.87±0.08 h) (Table 1).

The values of $t_{1/2\beta}$, CL_B, MRT and $AUC_{0\rightarrow\infty}$, determined by the non-compartmental pharmacokinetic analysis (Table 2) were equal or similar to those obtained by the compartmental method (Table 1).

Aqueous humour tobramycin levels increased rapidly after the i.v. administration and were detected for 24 hours (0.013±0.003 µg/mL by hour 24). The maximum antibiotic concentration in the aqueous humour was 2.24±0.29 µg/mL by hour 2 (t_{max}=2 h). The biological half-life of tobramycin in aqueous humour was 3.04 ± 0.21 h. $AUC_{0\rightarrow\infty}$ was 14.43 ±

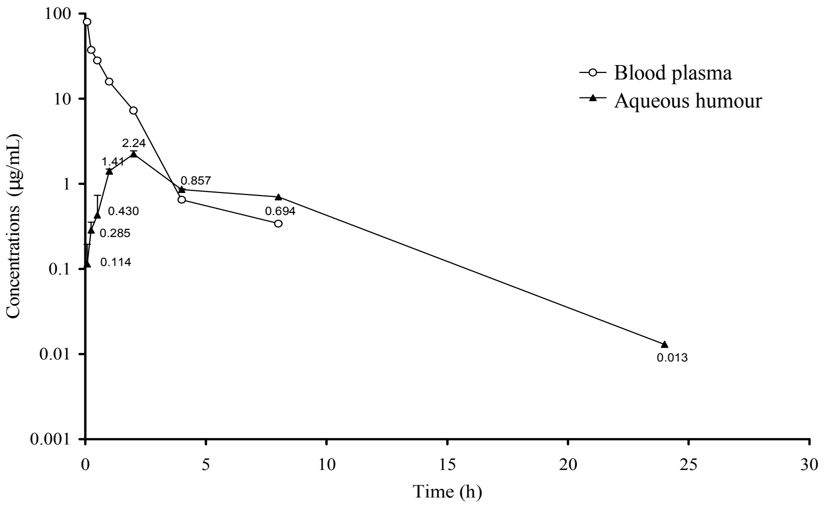

Fig. 1. Tobramycin concentrations in blood plasma and aqueous humour in dogs following a single intravenous adminsitration of 3 mg/kg tobramycin sulfate.

Table 1. Some pharmacokinetic parameters of tobramycin in blood plasma following a single intravenous administration in dogs at a dose of 3 mg/kg

Compartmental analysis		*Non-compartmental analysis*	
Parameters (units)	Mean ± SEM	Parameters (units)	Mean ± SEM
β (h^{-1})	0.837 ± 0.09	β (h^{-1})	0.580 ± 0.06
α (h^{-1})	11.970 ± 1.83	$t_{1/2\beta}$ (h)	1.30 (1.37)$^{\bullet}$ ±0.20
K_{el} (h^{-1})	2.530 ± 0.44	MRT (h)	1.06 ± 0.09
$t_{1/2\alpha}$ (h)	0.09 (0.09)$^{\bullet}$ ± 0.16	V_{ss} (L/kg)	0.06 ± 0.01
$t_{1/2\beta}$ (h)	0.87 (0.93)$^{\bullet}$ ± 0.09	$Vd_{(area)}$ (L/kg)	0.10 ± 0.02
MRT (h)	1.09 ± 0.12	Cl_B (mL/min/kg)	0.91 ± 0.20
V_{ss} (L/kg)	0.06 ± 0.01	$AUC_{0\rightarrow 8h}$ (μg.h/mL)	56.67 ± 5.03
Cl_B (mL/min/kg)	0.97 ± 0.08	$AUC_{0\rightarrow\infty}$ (μg.h/mL)	57.59 ± 5.32
C°_{p} (μg/mL)	132.03 ± 0.02	$AUMC_{0\rightarrow\infty}$ (μg.h^2/mL)	60.52 ± 6,20
$AUC_{0\rightarrow\infty}$ (μg.h/mL)	54.05 ± 4.68	r	0.876 ± 0.02

• – harmonic mean; α – hybrid rate constant in the distribution phase; β – hybrid rate constant in the elimination phase; $t_{1/2\alpha}$ – distribution phase half-life time; $t_{1/2\beta}$ – elimination phase half-life time; k_{el} – elimination constant; MRT – mean residence time; $Vd_{(area)}$ – area volume of distribution (area under the curve to ∞);. V_{ss}– volume of distribution at steady-state; Cl_B – total body clearance; $C°_p$ – concentration at t=0; $AUC_{0\rightarrow\infty}$ – area under the concentration vs time curve from 0 to ∞; r – correlation coefficient.

Table 2. Some pharmacokinetic parameters of tobramycin in aqueous humour following a single intravenous administration in dogs at a dose of 3 mg/kg

Parameters (units)	Mean ± SEM	Parameters (units)	Mean ± SEM
β (h^{-1})	0.233 ± 0.020	$AUMC_{0\rightarrow\infty}$ (μg.h^2/mL)	77.85 ± 13.12
$t_{1/2\beta}$ (h)	3.04 (3.18)$^{\bullet}$ ± 0.134	C_{max} (μg/mL)	2.24 ± 0.29
MRT (h)	5.24 ± 0.28	T_{max} (h)	2.00 ± 0.00
$AUC_{0\rightarrow 24h}$ (μg.h/mL)	14.38 ± 2.02	r	0.962 ± 0.14
$AUC_{0\rightarrow\infty}$ (μg.h/mL)	14.43 ± 2.02		

• – harmonic mean; β – hybrid rate constant in the elimination phase; $t_{1/2\beta}$ – elimination phase half-life time; k_{el} – elimination constant; MRT – mean residence time; $AUC_{0\rightarrow\infty}$ – area under the concentration vs time curve from 0 to ∞; $AUMC_{0-\infty}$ – area under the moment concentration vs time curve from 0 to ∞; r – correlation coefficient.

2.02 μg.h/mL that was about 1/4 of blood plasma $AUC_{0\to\infty}$ values in treated dogs ($AUC_{0\to\infty}$= 54.05±4.68 μg.h/mL and $AUC_{0\to\infty}$= 57.59±5.32 μg.h/mL) (Tables 1 and 2).

Plasma and aqueous humour tobramycin levels (Fig. 1) suggested that following the intravenous application of the drug, aqueous humour concentrations were higher than those in plasma by hour 4 (0.857±0.081 μg/mL and 0.647±0.084 μg/mL respectively). The ratio of aqueous humour $AUC_{0\to\infty}$ to blood plasma $AUC_{0\to\infty}$ was 0.2506. The corresponding ratio by post treatment hour 8 was 0.2537.

DISCUSSION

The results obtained following the intravenous administration of tobramycin showed that the dynamics of blood concentrations corresponded to a two-compartmental pharmacokinetic model. The presence of the antibiotic in *blood plasma* following this route of administration was characterized with a rapid initial phase of distribution, followed by a relatively slow elimination phase.

The biological half-life of blood plasma tobramycin levels in the dog, established in our studies (1.30±0.20 h and 0.87±0.09 h by the non-compartmental and compartmental method respectively) was shorter compared to that in llamas (3.68±1.26 h), camels (3.15 h) and humans (2.04 h and 2.15 h) and similar to that in cats (1.15 h), rabbits (1.23 h), pigeons (0.82 h) (Regamey *et al.*, 1973; Péchere *et al.*, 1976; Blouin *et al.*, 1979; Jernigan *et al.*, 1988; Brown and Riviere, 1991; Hadi *et al.*, 1994; Christinsen *et al.*, 1996; Dimitrova *et al.*, 1998; Lashev and Dimitrova, 2002). The *plasma MRT* values of treated dogs, determined by the compartmental and non-compartmental method (1.09 h and 1.06 h) are comparable to MRT values in llamas – 5.53 h (Christinsen *et al.*, 1996), camels – 4.23 h (Hadi *et al.*, 1994), cats – 1.5 h (Jernigan *et al.*, 1988) and 1.8 h (Brown & Riviere, 1991), rabbits – 1.58 h (Lashev and Dimitrova, 2002) and pigeons – 1.19 h (Dimitrova *et al.*, 1998). The steady-state volume of distribution of tobramycin in dogs suggests that its intravenous adminsitration, compared to humans and other animals species, resulted in a weaker penetration and distribution in body tissues and fluids compared to those in llamas (Vss=0.288 L/kg) (Christinsen *et al.*, 1996), camels ($Vd_{(area)}$=0.245 L/kg and Vss=0.288 L/kg) (Hadi *et al.*, 1994), humans – $Vd_{(area)}$=0.442 L/kg (Blouin *et al.*, 1979; Regamey *et al.*, 1972), rabbits – $Vd_{(area)}$=0.350 L/kg and Vss =0.470 L/kg (Lashev and Dimitrova, 2002) and pigeons – $Vd_{(area)}$=0.292 L/kg (Dimitrova *et al.*, 1998).

The pharmacokinetic parameters of tobramycin in aqueous humour were indicative for early high antimicrobial concentrations in the eyes of treated dogs. The C_{max}, t_{max}, $t_{1/2\beta}$ and MRT values showed a good diffusion and prolonged presence of the antiiotic in tobramycin–treated dogs. Following an i.v. administration, the antibiotic passes the haemoophthalmic barrier and 5 min after the application was discovered in aqueous humour in detectable concentrations, that were sustained up to post treatment hour 8 in levels higher that the MIC of *Staphylococcus aureus*, *Pseudomanas aeruginosa*, *Proteus spp. Actinobacillus spp., Bordetella canis* (Riviere and Spoo, 1995; Anonymous, 1997). This fact is of importance for veterinary ophthalmology in the treatment of infectious diseases of the anterior or posterior ocular camera, caused by highly susceptible bacterial agents.

In conclusion, the pharmacokinetic parameters of tobramycin after an intravenous administration in dogs at the dose of 3 mg/kg allow its use as a chemotherapeutic agent for systemic application at 8 h intervals .

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