



GENTAMICIN DISPOSITION IN CEREBROSPINAL FLUID (CSF) AND AQUEOUS HUMOUR IN HEALTHY DOGS

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ABSTRACT

Aim: The aim of the present study was to determine the penetration of aminoglycoside antibiotic gentamicin in aqueous humour (AH) and cerebrospinal fluid (CSF).

Material and methods: Six crossbred clinically healthy dogs were used. A single bolus dose of Gentamicin 4% sterile solution for injections (Sopharma, Sofia, Bulgaria) was administered intravenously into the cephalic vein at a dose rate of 4 mg/kg. Antibiotic concentrations in plasma, CSF and AH were determined by microbiological assay.

Results: Gentamicin was found in all studied fluids till 8 hrs after drug administration. Rate constants which characterized distribution rate of the drug from plasma to AH ($1.09 \pm 0.39 \text{ h}^{-1}$) and from plasma to CSF ($1.84 \pm 1.91 \text{ h}^{-1}$) were higher than these which described antibiotic disposition in the opposite direction, namely from AH to plasma ($0.65 \pm 0.37 \text{ h}^{-1}$) and from CSF to plasma ($0.30 \pm 0.14 \text{ h}^{-1}$).

Conclusion: The data from the current study with healthy animals show that gentamicin penetrates CSF to a limited extent after i.v. administration and that it is not advisable to use it for treatment of inflammation of central nervous system. The antibiotic penetrates at higher extent in AH and measured concentrations reached therapeutic levels. These results should be validated in further clinical studies.

Key words: disposition of gentamicin, aqueous humour, cerebrospinal fluid, dogs.

INTRODUCTION

Aminoglycoside antibiotics are widely used in veterinary practice because of their activity against many Gram-negative microorganisms. Gentamicin is a highly polar drug with poor tissue penetration and low values of volume of distribution (1). The degree of binding to plasma proteins is low (approximately 10%). The absorption is rapid and the absolute bioavailability after parenteral extraveneous administration is between 70 and 100%. The pharmacokinetics (PK) of gentamicin has been investigated in a number of animal species, including dogs (1, 2, 3). However, its penetration in AH and CSF is hardly investigated and mainly in humans (4). PK-pharmacodynamic (PD) modelling has

been used to optimize antimicrobial dosage regimens, to improve the outcome and reduce the selection of resistant mutants (5). The PK-PD index for aminoglycosides with the most predictive value about clinical outcome is the

ratio of peak plasma concentration to MIC (6). According to our knowledge, information about this index for aqueous humour and CSF in dogs is virtually lacking.

The aim of these preliminary studies was to determine the penetration of gentamicin in aqueous humour and CSF and to estimate PK-PD indices.

MATERIALS AND METHODS

Drug

Gentamicin as a 4% sterile solution for injections (Sopharma, Sofia, Bulgaria) was used.

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Experimental animals

The experiments were performed with 6 crossbred clinically healthy dogs at the age of 3–5 years weighing 18–25 kg, reared in individual cages with an area of 1.5 m² and height of 2.2 m. During the 14-day period of adaptation, each animal was provided with food in individual bowl and had free access to water. The housing conditions were uniform.

Experimental design

In the beginning of the experiment, all animals were submitted to xylazine-ketamine anaesthesia using the following protocol: atropine sulfate s.c. (Atropinum sulfuricum 0,1%, Sopharma, Sofia, Bulgaria) at 0.04 mg/kg; xylazine i.m. (Xylazin 2%, Alfasan International B.V.) at 1.0 mg/kg; ketamine i.m. (Ketaminol 10%, Intervet International B.V.) at 10 mg/kg. When obtaining blood samples after the first hour, the analgesia was achieved by local infiltration of lidocaine (Lidocain 2%, Sopharma, Sofia, Bulgaria).

After the administration of ketamine, a single bolus dose of Gentamicin 4% sterile solution for injections (Sopharma, Sofia, Bulgaria) was administered intravenously into the cephalic vein at a dose rate of 4 mg/kg. The samples were obtained at the following time intervals: 0.083, 0.25, 0.50, 1, 2, 4, 8 and 24 h after antibiotic administration. Blood was sampled from the cephalic vein in heparinized tubes, blood plasma was separated after centrifugation at 10 min at 3500 rpm and deeply frozen at –18°C.

Aqueous humour (300 µL) was obtained via puncture of the anterior ocular chamber with a 27G sterile injection needle (Molina krepost PLC, Veliko Tarnovo, Bulgaria) and sterile 2 mL syringe. The samples were stored in Eppendorf tubes at –18 °C.

Cerebrospinal fluid samples of 300 µL were obtained by puncture of the cerebellomedullary cistern with a 22G spinal needle (Sherwood Medical, U.S.A.). The samples were stored in Eppendorf tubes at –18 °C until analysis.

Drug assay

The concentrations of gentamicin in plasma, AH and CSF were determined using *Bacillus subtilis* ATCC 6633 as a test organism (7). Hottinger agar for streptomycin was used as a nutrient medium (NCIPD, Sofia, Bulgaria). The standard solutions were prepared in plasma, aqueous humour and CSF collected from untreated dogs. The limit of quantification for gentamicin was 0.048 µg/mL. The concentrations of the standard solutions were 25, 12.5, 6.25, 3.12, 1.56, 0.78,

0.39, 0.195, 0.098, 0.048 and 0.024 µg/mL. The linearity (presented as r^2) for gentamicin was 0.990, and the test for lack of fit was without statistical significance. The intra-assay and the interassay coefficients of variation (CV) for gentamicin were 6.6 and 10.2, respectively.

Pharmacokinetic analysis

Pharmacokinetic parameters for plasma were calculated with two-compartmental analysis and these for CSF and HA were estimated with one-compartmental analysis. A non-compartmental analysis was also used. The pharmacokinetic parameters of gentamicin were calculated with the WinNonlin 4.0.1 software (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA). The pharmacokinetic parameters were calculated individually for each animal and presented as mean ± standard deviation. The following parameters were determined: the hybrid rate constant for the elimination phase (β), obtained from the terminal part of the drug-concentration time curve; the elimination half-life ($t_{1/2\beta}$), distribution rate constants for transferring the drug from plasma to AH and back (k_{12} and k_{21}), distribution rate constants for transferring the drug from plasma to CSF and back (k_{13} and k_{31}), the area under the drug concentration-time curve ($AUC_{0-\infty}$), calculated according to the linear trapezoidal rule with extrapolation to infinity. Also, the total body clearance (Cl_B), the maximum plasma concentrations (C_{max}), the time of C_{max} (T_{max}), the mean residence time (MRT) were determined. Values of relation of $AUC_{0-8h(AH/CSF)}/AUC_{0-8h(plasma)}$ were also estimated.

Statistical analysis

The PK parameters of gentamicin were presented as mean (SD). They were evaluated for significance with the STATISTICA 6.1 computer programme (Statistica for Windows, StatSoft, Inc., Tulsa, OK, USA, 1984–2002). Statistically significant differences were determined using the Wilcoxon test.

RESULTS

The mean concentration-time curves of gentamicin in plasma, CSF and AH are presented in Fig. 1. According to the model-discriminating criteria used, the plasma concentration–time data were best fitted by a two-compartment open model. The data for CSF and AH were analyzed by one-compartmental model. A summary of the kinetic parameters is given in Table 1.

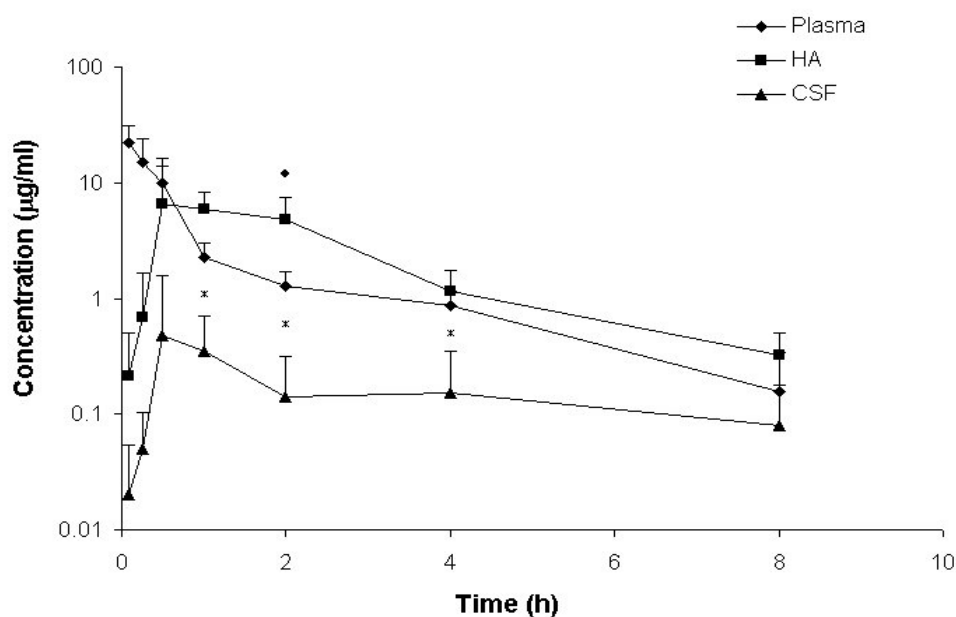


Fig. 1. Gentamicin concentrations (mean±SD) in plasma, aqueous humour (AH) and cerebrospinal fluid (CSF) after intravenous administration in dogs (n=6) at a dose rate of 4 mg/kg. * - Statistically significant ($p<0.05$) differences between plasma and cerebrospinal fluid concentrations; • - Statistically significant differences ($p<0.05$) between plasma aqueous humour concentrations

Table 1. Pharmacokinetic parameters of gentamicin (mean±SD) administered intravenously at a dose rate of 4 mg/kg in healthy dogs (n=6).

Pharmacokinetic parameters (units)	Plasma	Aqueous Humour	Cerebrospinal fluid
<i>Compartmental analysis</i>			
k_{10} (h^{-1}) / k_{21} , k_{31} /	1.83 ±0.86	0.65 ±0.37	0.30 ±0.14
k_{01} (h^{-1}) / k_{12} , k_{13} /		1.09 ±0.39	1.84 ±1.91
Cl_B ($\text{ml.h}^{-1}.\text{kg}^{-1}$)	245.97 ±65.63		
<i>Non-compartmental analysis</i>			
$AUC_{0-\infty}$ ($\mu\text{g.h.ml}^{-1}$)	17.58 ±5.87	19.55 ±8.58	2.18 ±2.02*
$t_{1/2\beta}$ (h)	1.77 ±0.98	2.17 ±1.72	4.58 ±2.10
C_{\max} ($\mu\text{g/ml}$)		9.78 ±7.72	0.78 ±1.00
T_{\max} (h)		1.00 ±0.55	1.92 ±1.63
MRT (h)	1.53 ±0.38	2.89 ±0.94*	5.82 ±3.00*
MAT (h)		1.35 ±1.15	4.22 ±3.05
$AUC_{0-8h}(\text{AH/CSF})/AUC_{0-8h}$		110.25 ±51.69	9.43 ±8.64
(plasma)			

$t_{1/2\beta}$ - terminal elimination half-life; k_{10} - rate constant for drug elimination from central compartment; k_{12} and k_{21} - distribution rate constants for transferring the drug from plasma to AH and back; k_{13} and k_{31} - distribution rate constants for transferring the drug from plasma to CSF and back; $AUC_{0-\infty}$ - area under the serum concentration-time curves from 0 h to ∞ ; AUC_{0-8h} - area under the serum concentration-time curves from 0 h to 8 h; MRT - mean residence time, MAT - mean absorption time; Cl_B - total body clearance; C_{\max} - maximum serum levels; T_{\max} - time of C_{\max} .

* - Differences are statistically significant ($p<0.05$) in comparison to plasma.

Statistically significant differences were observed between the values of AUC for plasma and CSF and for MRT in plasma and the other two fluids, only. Relatively big inter-individual variations were observed and statistically significant differences between the values of other pharmacokinetic parameters were not found (Table 1).

DISCUSSION

Pharmacokinetics of gentamicin in the dog has been described previously (3) but according to our knowledge its disposition in CSF and humor aqueous in this animal species was studied to limited extent. Two-compartmental model was used because of sensitivity of our method and the third phase could not be described. In this study, the pharmacokinetic parameters estimated in dogs are similar to those previously reported (2, 8, 9). Low penetration of gentamicin in tissues and its high affinity to concentrate in the renal cortex to a far greater extent than in other tissues is well described (3). This fact suggests that the peripheral tissue compartment is heterogeneous and distribution of the antibiotic could not be predicted on the basis of plasma concentrations only. Therefore, the current study was designed to determine the penetration of gentamicin in CSF and aqueous humour.

Values of the distribution constants indicate that the rate of gentamicin passage from plasma to aqueous humour was higher than those in the opposite direction. The high AUC value, percentage of penetration and the significantly longer MRT values suggest that gentamicin has a tendency to reside in this compartment. C_{max} values in the canine eye were reached within an hour and could ensure achievement of therapeutic levels against sensitive microorganisms after i.v. administration of gentamicin. The measured concentrations of gentamicin in aqueous humour reflect the penetration of drug molecules after systemic administration mainly across the posterior chamber wall and then, their distribution in both anterior chamber and the vitreous body (10). Gentamicin distribution in canine eye differs than those observed in human beings. This drug reached subtherapeutic levels (<0.2 $\mu\text{g/ml}$) in the uninfected human vitreous body following systemic administration (4). A possible reason for the observed differences could be the used pharmaceutical

drug formulations which contain mainly C1, C1a, C2 and a number of minor components in different proportions. The different fractions of gentamicin have different pharmacokinetic characteristics (11).

The choice of antibiotics for treatment of infections of CSF must be based on the antibacterial spectrum of the drugs considered but also on their ability to penetrate into the cerebrospinal fluid or the brain tissue. This study is concentrated on the possibilities of gentamicin to pass blood-CSF barrier. Very low concentrations of this antibiotic were detected in the CSF of dogs in our study. Distribution constants indicate that the penetration rate of this antibiotic into CSF was much faster than the rate of elimination. Thus, it should be expected that gentamicin will persist in CSF longer than in blood. This is supported by the observed tendencies for longer elimination half-life and significantly higher value of MRT for the CSF compared to those for plasma and by the high value of MAT. Maximum CSF concentration could be expected 2 hrs after i.v. drug administration. Altogether, these data suggest that an increase of CSF concentration could be expected after repetitive gentamicin administration. From clinical point of view, it is also important to take into account that gentamicin penetration in the CSF is rather low, around 10 % till the 8th h after treatment. It should be acknowledged that method of comparing AUC values in plasma and CSF could not give complete information about the disposition of gentamicin in CSF because other factors such as active transport could play a role (12).

The efficacy of aminoglycoside drugs correlates with the achievement of C_{max}/MIC equal to 8–10 (13, 14). The data obtained in our study after i.v. administration of gentamicin for CSF show that this value could be achieved for extremely sensitive microorganisms with MIC less than 0.08 $\mu\text{g/ml}$. The results for aqueous humour indicate that the desired breakpoint of C_{max}/MIC index could be achieved for sensitive microorganisms with MIC values lower than 1 $\mu\text{g/ml}$. These results should be discussed carefully since the function of blood-CSF and blood-eye barriers could be changed during the inflammation and changes in penetration profile of gentamicin could be expected. Therefore, these results need to be validated in further clinical trials. It is also important to test the possibilities for achievement of desirable pharmacokinetic-

pharmacodynamic indices and clinical results after topical administration of gentamicin in eye infections.

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