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Pharmacokinetics of Amikacin after Intravenous Intramuscular and Subcutaneous Administration in German Shepherd Dog

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Abstract: The purpose of the study was to compare the pharmacokinetics of amikacin in German Shepherd dogs following single intravenous intramuscular and subcutaneous injections. Amikacin was administrated by a randomized three cross-over design after single intravenous (10 mg kg^{-1}) intramuscular (10 mg kg^{-1}) and subcutaneous injections (10 mg kg^{-1}) in 9 healthy German Shepherd dogs. The concentrations of Amikacin in plasma were determined by LC-MS/MS and the concentration-time data were analyzed with 3 p97 program. It's best to fit the amikacin concentration-time data to two-compartment open model after single i.v. dosing. The main pharmacokinetic parameters were as follows: $T_{1/2\beta}$ = (1.61±0.07) h, V_c = (0.10±0.01) L kg^{-1}, $CL_{(s)}$ = (0.08±0.01) l kg^{-1} ·h, AUC = (129.15±6.74) μg mL^{-1} ·h. A two-compartment model with first order absorption best described the drug concentration-time data after single i.m. and s.c. in healthy dogs. The main pharmacokinetic parameters were as follows: $T_{1/2\alpha}$ = (0.49±0.07) h and (0.64±0.08) h, respectively; $T_{1/2\beta}$ = (1.40±0.12) h and (1.78±0.15) h, respectively; T_{peak} = (0.79±0.09) h and (0.89±0.07) h, respectively; C_{max} = (44.59±2.10) μg mL^{-1} and (31.42±1.39) μg mL^{-1}, respectively; F = (91.3±6.6) and (81.0±5.6)%, respectively.

Key words: Amikacin, German Shepherd dog, pharmacokinetics, LC-MS/MS, plasma injections, China

INTRODUCTION

Amikacin (AMK), an semisynthetic aminoglycoside antimicrobial has a broad spectrum of antibacterial activity including gram-positive and negative bacteria (Lorena *et al*., 2010), it is one of the most widely-used antimicrobial, approved by FDA in 1976. Amikacin was concentration-dependent and it cannot be taken up in oral administration but when administrated in intramuscular, amikacin have a rapid and complete absorption, a high bioavailability and a short time to achieve peak concentration (Abo, 1999; Wasfi *et al*., 1999; Errecalde *et al*., 2001; KuKanich and Coetzee, 2008). In now days, amikacin injections were used in clinical, the preparation released by a mode of kinetic, the plasma concentration changed dramatically and which maybe exceeded the minimum toxic concentration and the maintain time of effective plasma concentration was short so, it needed a repeated administration. Which was stimulated in animals strongly and harmful of their growth and it might lead to the development of bacteria that are resistant to antibiotics. The purpose of the study was to compare the pharmacokinetics of amikacin in German Shepherd dogs with single intravenous intramuscular and subcutaneous administration which may contribute to its clinical application.

MATERIALS AND METHODS

Animals: Nine healthy adult German Shepherd dogs (male and female randomly) weighing (29.3±1.65) kg were used in the study, aged nearly 2 years. All dogs were confirmed healthy by physical examinations, complete blood counts and serum chemistry profile, they were provided by Dog Base of Guangdong Provincial Police Department.

Chemicals: Unless indicated, reagent-grade was used. Amikacin sulfate (≥98.5% purity) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), amikacin sulfate injections were supplied by Guangdong Succhi Pharmaceutical Ltd. Guangzhou, Guangdong, China and Heptafluorobutyric Acid (HFBA) was obtained from Fluka, Buchs, Switzerland. Acetonitrile and methanol (HPLC grade) was obtained from Fisher (Bar-Bel, France), other analytical-reagent grade chemicals were made in china and milli-Q water was used throughout.

Administration and sampling: Dividing nine healthy German Shepherd dogs into three groups, amikacin was administrated by a randomized three period cross-over design following single intravenous intramuscular and subcutaneous injections (10 mg kg^{-1}). Each experiment

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was followed by seven days. Blood samples were collected at 5, 10, 15, 30 min and at 1, 2, 3, 4, 6, 8, 12 and 24 h after drug administration. Then, the blood was centrifugated at 3000 rpm for 10 min and the plasma was kept in plastic tubes at -20°C until analysis.

Sample preparation: The blood samples were thawed and shacked and 0.25 mL samples were took into a 2 mL centrifuge tube, volumes of 0.5 mL Trichloroacetic Acid (TCA, 5%) was added and mixed by vortexing for 3 min then the blood was centrifugated at 15000 g for 10 min.The supernatant was transferred to a 15 mL plastic tubes, the extraction step was repeated, the supernatant of twice extraction was added into the 15 mL plastic tubes together and volumes of 3 mL ethanol was added into it for accelerating dryness. The elute was evaporated to dryness under a nitrogen stream at 40°C and then dissolved with 10 mM HFBA and vortexed to completely dissolve, 1 mL of the solution was transferred to a 2 mL centrifuge tube, 0.5 mL hexane were added and mixed by vortexing after centrifugated at 15000 g for 10 min, the water-based phase was filtered through nylon centrifuge filter (0.22 μm) for detection.

Conditions of LC-MS/MS: LC was performed using an Agilent 1200 HPLC System (Waldbronn, Germany) equipped with an automatic degasser, a quaternary pump and an autosampler. Chormatographic separation was carried out using a Hypersil C_8 (5 μm, 2.1×150 mm, Dalian, China) at (35±1)°C. The mobile phase A was 10 mM HFBA/acetonitrile and mobile phase B was 10 mM HFBA/water. The flow-rate of mobile phase was maintained at 250 μL min^{-1} and the injection volume was 5 μL. A gradient elution program was started with 20% A increased to 90% of A at 2.5 min, kept at 90% of A for 3 min, returned to initial composition at 5.5 min and held for 4.5 min to equilibrate the column.

The HPLC system was connected to an API 4000 triple quadrupole tandem Mass spectrometer, Applied Biosystems, USA, equipped with ESI ion source. The optimization of mass condition was achieved on infusing injection of each compound separately at a flow rate of 45l min^{-1}. The source block temperature was set at 600°C in the positive ion mode with a capillary voltage 5000 V. Nitrogen gas was used as nebulizer gas (40 psi), auxiliary gas (45 psi), collision gas (7 psi) and curtain gas (20 psi). Detection was operated in the Multiple Reaction Monitoring (MRM) modes. The higher intensity transition was selected for quantitative purpose and the resolution was set at unit (0.7 U). The instrument control and data acquisition were carried out by the analyst 1.5 Software.

Table 1: Molecular weight and optimized MS parameters for AMK using ESI^+ mode

	Ion ($m z^{-1}$)						
Compound	Precursor	Product	Dwell time (msec)	CE (eV)	DP (V)	EP (V)	CXP (V)
AMK	586.1	163.1[a]	200	80	10	48	12
		425.1	200	80	10	40	12

[a]Selected for quantification ion

Mass parameters for each analyte including precursor ion (Q1), product ion (Q3), dwell time, Declustering Potential (DP), Entrance Potential (EP), Cell Exit Potential (CXP), Collision Energy(CE) were shown in Table 1. In the condition above, amikacin in the plasma could be separated from impurities and the peak time was 7.01 min.

Data analysis: The pharmacokinetic analysis of amikacin was performed using 3 p97 Pharmacokinetic Software. Amikacin mean concentration-time datas were analyzed by compartment model using the computer program. It's best to fit the amikacin concentration-time data to two-compartment open model after single i.v. dosing and a two-compartment model with first order absorption best described the drug concentration-time data after single i.m. and s.c. administration. A linear relationship exited in the calibration curve over the range of 0.05-2 μg mL^{-1} which always yielded a correlation coefficient exceeding 0.997. The within-run and inter-run precision and recovery for amikacin was 4.03~6.66, 6.11~8.13 and 79.29~109.17%, respectively. The limit of detection was 0.02 μg mL^{-1} and the limit of quantification was 0.05 μg mL^{-1}.

RESULTS AND DISCUSSION

After a single i.v., i.m. and s.c. administrations in German Shepherd dogs, AMK concentrations were shown in Table 2, AMK concentration-time curves were showed in Fig. 1-3 and pharmacokinetic parameters were shown in Table 3. The plasma concentrations decreased rapidly through 1 h after i.v. administration, amikacin concentration was 0.39 μg mL^{-1} at 12 h and the concentrations were below the limit of detection after 24 h (Fig. 1). Following single i.m. administration, the peak concentration of amikacin of 44.59 μg mL^{-1} was found at 0.79 h after administration, the concentration was 0.19 μg mL^{-1} after 12 h, the concentrations were below the detectable limit after 24 h and the absolute bioavailability was (91.32±6.58%) (Fig. 2). After single s.c. dosing, the peak concentration of amikacin of 31.42 μg mL^{-1} was found at 0.89 h after dosing, the concentration was 0.45 μg mL^{-1} after 12 h, the concentrations were below the detectable limit after 24 h and the absolute bioavailability was (81.02±5.55%) (Fig. 3).

Table 2: AMK concentrations in the plasma following single i.v., i.m. and s.c. dosing (10 mg mL^{-1}) (X±SE, n = 9) (μg mL^{-1})

Time after administration (h)	Concentrations in the plasma (μg mL^{-1})		
	i.v	i.m	s.c
0.083	81.44±2.42	9.42±1.01	4.06±0.40
0.167	67.61±1.98	19.76±1.51	9.88±1.00
0.25	55.58±1.41	26.59±2.06	17.50±1.73
0.5	44.71±1.45	46.00±4.53	30.59±2.18
1	33.23±1.45	41.64±2.80	32.62±3.06
2	25.67±1.82	24.90±1.48	24.97±2.49
3	14.98±1.46	15.50±1.42	16.40±1.95
4	8.53±0.85	10.03±1.58	10.75±0.93
6	4.19±0.54	3.79±0.96	4.34±0.70
8	1.72±0.22	1.91±0.54	2.28±0.60
12	0.39±1.56	0.19±0.06	0.45±0.21
24	ND	ND	ND

ND: No Detected

Table 3: Pharmacokinetis parameters of AMK following single i.v., i.m. and s.c. dosing (10 mg mL^{-1}) (X±SE, n = 9)

Parameters	i.v.	i.m.	s.c.
K_a (h^{-1})	-	2.20±0.24	2.09±0.21
T_{Lag} (h^{-1})	-	0.03±0.01	0.05±0.01
$V/F_{(c)}$ (L kg^{-1})	0.10±0.01	0.13±0.01	0.19±0.01
$T_{1/2\alpha}$ (h^{-1})	0.12±0.02	0.49±0.07	0.64±0.08
$T_{1/2\beta}$ (h^{-1})	1.61±0.07	1.40±0.12	1.78±0.15
$T_{1/2Ka}$ (h^{-1})	-	0.42±0.07	0.37±0.04
K_{21} (h^{-1})	3.69±0.46	1.32±0.28	1.05±0.17
K_{10} (h^{-1})	0.86±0.06	0.76±0.06	0.55±0.04
K_{12} (h^{-1})	3.10±0.60	0.14±0.05	0.17±0.08
AUC (μg mL^{-1} ·h)	129.15±6.74	117.04±9.44	105.05±9.37
$CL_{(s)}$ (L kg^{-1} ·h)	0.08±0.01	0.09±0.01	0.10±0.01
$T_{(peak)}$ (h^{-1})	-	0.79±0.09	0.89±0.07
$C_{(max)}$ (μg mL^{-1})	-	44.59±2.10	31.42±1.39
F (%)	-	91.32±6.58	81.02±5.55

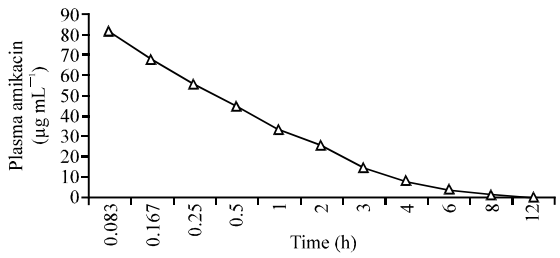

Fig. 1: AMK concentration-time curves following single i.v. dosing (10 mg kg^{-1})

In a word, the pharmacokinetics of amikacin in German Shepherd dog was as follows; intravenous dosing with a rapid and wide distribution intramuscular administration and subcutaneous injections with a rapid absorption, a short half-life of elimination and a high bioavailability and it had a shorter time to achieve peak concentration, a higher peak concentration and a higher bioavailability by intramuscular administration.

From the molecular structure of amikacin which was known that amikacin was polar, thermo labile and water-soluble without a distribution coefficient between water and oil and it was not volatile and lack of UV absorption characteristics so, it was difficult to detect with UV

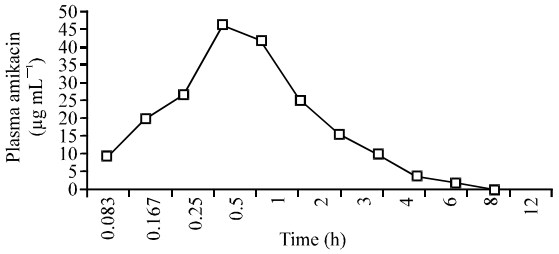

Fig. 2: AMK concentration-time curves following single i.m. dosing (10 mg kg^{-1})

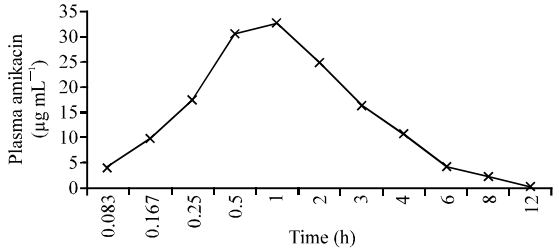

Fig. 3: AMK concentration-time curves following single s.c. dosing (10 mg kg^{-1})

detector. But a substance formed when its lively group (Amino) reacted with derivatization reagent, the substance could be detected by UV or fluorescence detector (Feng *et al*., 2001; Santos *et al*., 2002; Nicoli and Santi, 2006).

The study had tried a method of Post-Column Derivatization, the chormatographic column was a Hypersil C_8 (4×150 mm, 5 μm), the samples were extracted with trichloroacetic acid then they were purified with sodium hydroxide, phosphate buffer and dichloromethane after derivatived with o-phthalaldehyde, the samples were extracted with 2-propanol. The mobile phase was Methanol: EDTA solution (69:31, V/V), the flow-rate was 1.5 mL min^{-1}, the excitation wavelength was 360 nm and the detection wavelength was 435 nm, the linear range was 0.5~10 μg mL^{-1}. But there was a low peak response of drug and a poor peak shape which did not meet the test requirements. Compared with the results of SARITOS B (Santos *et al*., 2002) the recovery was lower and the limit of detection was higher in the study. Which showed that it was tedious, time-consuming, low precision and poor repeatability by derivatization.

LC-MS/MS was applied widely in detection of Aminoglycosides (Kaufmann and Maden, 2005; Babin and Fortier, 2007; Zhu *et al*., 2008). The study established a LC-MS/MS and heptafluorobutyric acid was used as a ion-pair reagent to increase the retention time of amikacin, 10% Trichloroacetic acid was used as extractant to increase the recovery, the liner range, recovery, limit of detection and limit of quantification in the study was similar with the reports earlier.

The study established a LC-MS/MS Method of detecting plasma amikacin concentrations in healthy German Shepherd dog which was simple, short time-consuming, high recovery and precision, it could meet the requirement of pharmacokinetics detection of amikacin in plasma. When amikacin was given intravenously in healthy German Shepherd dog, it's best to fit the amikacin concentration-time data to two-compartment open model. Compared to camels (Wasfi *et al*., 1999) and lactating goats (Abo, 1999) German Shepherd dog had a shorter half-life of absorption and a shorter half-life of elimination but it had a bigger volume of distribution. Moreover, the $CL_{(s)}$ of German Shepherd dog was more than lactating goats (Abo, 1999) and calves (Errecalde *et al*., 2001) smaller than Greyhound, Beagle dogs (KuKanich and Coetzee, 2008) and camels (Wasfi *et al*., 1999). The results showed that amikacin had a rapid and wide distribution and a rapid elimination in the plasma after intravenous administration.

Following single i.m. administration, a two-compartment model with first order absorption best described the drug concentration-time data. The German Shepherd dog had a longer half-life of absorption than camels and a similar half-life of elimination with camels (Wasfi *et al*., 1999). However, German Shepherd dog had a higher peak concentration when compared to calves (Errecalde *et al*., 2001), camels (Wasfi *et al*., 1999) and lactating goats (Abo, 1999) and the bioavailability was the same as camels (Wasfi *et al*., 1999) and maybe all the differences were associated with administration dose and animal species. The results suggested that amikacin had a rapid and complete absorption, a high peak concentration and bioavailability which played a significant role in clinical application.

After single s.c. dosing, a two-compartment model with first order absorption was best fitted with the concentration-time data. Contrasted to the results of (KuKanich and Coetzee, 2008) the $T_{1/2\alpha}$ and $T_{1/2\beta}$ was longer than Greyhound and Beagle dogs but the body clearance rate and the volumes of distribution were smaller than Greyhound and Beagle dogs which suggested that it had a more rapid and complete absorption and a slower rate of elimination. Moreover, the time to achieve peak concentration was shorter and the peak concentration was higher in German Shepherd dogs than Greyhound and Beagle dogs.

CONCLUSION

The pharmacokinetics of amikacin after intramuscular administration was different from subcutaneous dministration, the $T_{1/2\alpha}$, $T_{1/2\beta}$, C_{max} and bioavailability was smaller after subcutaneous administration this means that after subcutaneous administration, the rate of absorption and elimination was slower, the peak concentration and the bioavailability was lower which showed that the effect of subcutaneous administration was worse, it maybe connected with delaying absorption in different injection site.

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