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Original Contribution

PHARMACOKINETICS AND ALLOMETRIC ANALYSIS OF SPECTINOMYCIN

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ABSTRACT

In this study were compared microbiological and HPLC methods for determination of spectinomycin pharmacokinetics in goats. The goats were subject to intravenous administration of spectinomycin at 20 mg/kg body weight. For the microbiological assay was used test microorganism *Sarcina lutea* ATCC 9341. Limit of quantification in the microbiological assay was 6.25 µg/mL and in the HPLC method was 0.1 µg/mL. Spectinomycin concentrations following microbiological assay were initially higher than HPLC-determined and gradually the differences decreased. During HPLC analysis spectinomycin was found until 12 h and during microbiological assay until 4 h. As a result in HPLC analysis the values of $V_{d(area)}$ (0.527 L/kg) , $t_{1/2\beta}$ (1.74 h) and Cl_B (3.512 mL/kg/min) were considerably higher in comparison with microbiological method ($V_{d(area)}$ - 0.147 L/kg , $t_{1/2\beta}$ - 0.8 h and Cl_B - 2.174 mL/kg/min). The data from method validation also showed the advantage of HPLC method. Because of that can be concluded that HPLC is more sensitive and accurate method for spectinomycin determination.

Regarding the allometric equation for elimination half-life ($t_{1/2\beta} = 1.19.W^{0.02}$) the values are showing lack of correlation to body weight. Volume of distribution allometric equation ($V_{d(area)} = 0.37.W^{0.96}$) and total body clearance allometric equation ($Cl_B = 1.92.W^{1.09}$) have high level of correlation to body weight (<0.001) and therefore can be used.

Key words: Spectinomycin, pharmacokinetics, allometry.

INTRODUCTION

Spectinomycin is an aminocyclitol antibiotic produced by *Streptomyces spectabilis*. It is closely related by chemical structure to the aminoglycosides, but lacks their ototoxic and nephrotoxic effects (1, 2). Spectinomycin is used for treatment of *Neisseria gonorrhoeae* infections and for *Pasteurella multocida* and *Mycoplasma* spp. infections in human and veterinary medicine, respectively (3, 4). It has bacteriostatic effect at therapeutic dosage and also a unique property - it exhibited higher *in vivo* activity than *in vitro* (5). Regardless of the rapid development of resistance spectinomycin can still be used with caution in the human and veterinary medicine for treatment of infections caused by susceptible microorganisms (6).

The allometric analysis is one of the valuable tools in the medicine for prediction of the appropriate dosage in species for which no specific pharmacokinetic data are available. It can be used also for comparison of drug disposition in the body (7, 8).

Despite of the long use in medicine few studies are available regarding the pharmacokinetics of spectinomycin. Studies of spectinomycin pharmacokinetics in goats are not done until now. The purpose of the present experiment is to compare two methods for determination of spectinomycin pharmacokinetics, as well as to use the available literature data to perform allometric analysis.

MATERIALS AND METHODS

Animals

The trial was conducted with 6 nonlactating female goats (Bulgarian white dairy goat breed). During the study the goats weighed 45.5 ± 2.7 kg (mean ± SD). Experimental animals were housed in outdoor conditions, according to the specific requirements of the species. They were fed on alfalfa hay and a concentrated grain of wheat ration. Water was supplied *ad libitum*.

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Experimental design

The experiment complied with the current laws of Republic of Bulgaria. Spectinomycin was provided in 100 ml vials (Ceva, France) as a 10% solution and was administered at 20 mg/kg b.w. The drug was injected intravenously as a single bolus in *v. jugularis*. Blood samples were withdrawn prior to and at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24h post medication from the collateral vein. They were kept at room temperature for 2h and the separated serum was stored for 48h at -20 ^{0}C until the microbiological and HPLC assay.

Microbiological method

A meat peptone agar (National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria) and test microorganism *Sarcina lutea* ATCC 9341 were used for quantitative determination of spectinomycin. The limit of quantification (LOQ) of spectinomycin in the microbiological assay was 6.25 μg/mL, r^2 = 0.9778, intra-assay CV = 7.14% and inter-assay CV = 7.55%.

HPLC assay

Spectinomycin was determined by HPLC method based on a modified method of Burton *et al*., 1991 (9). The procedure for determining the concentrations of spectinomycin by HPLC methods started with mixing 100 μL serum + 400 μL of 3% trifluoroacetic acid in acetonitrile. After mixing the sample was centrifuged at 3200 x g for 5 min. To a 250 μL aliquot of the supernatant, 200 μL of 5 mg/mL solution of 2.4 dinitrophenylhydrazine in acetonitrile was added. The content was mixed, then heated at 70^{0}C for 1 h. Afterwards the sample was cooled on ice for 2 min and 30 μL of acetone was added. Additional mixing was applied and then another heating at 70^{0}C for 10 min was performed. After cooling on ice, the sample was purified and stored at room temperature until it was subjected to HPLC analysis. The limit of quantification (LOQ) of spectinomycin was 0.1 μg/mL, r^2 = 0.99975 and inter-assay CV = 5.3%.

Pharmacokinetic analysis

The pharmacokinetic analysis was done with the WinNonLin 4.0.1. (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA) software. Serum concentrations curves vs. time were interpreted by a two compartmental open model selected according Akaike's criterion. A bi-exponential equation was selected for the intravenous administration:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}$$

where $C(t)$ represent spectinomycin serum concentration at any time t and α and β are the slopes of the first and second phases of spectinomycin serum disposition.

Allometric analysis

Allometric analysis of pharmacokinetic parameters elimination half-life ($t_{1/2\beta}$), volume of distribution ($V_{d(area)}$) and total body clearance (Cl_B) was performed using data from published researches. Only data from intravenously administered drugs to healthy animals were used and the matrices of interest were serum or plasma. Data regarding body weights were collected from the respective papers. All values are calculated on the basis of the published value of pharmacokinetic parameters versus body weighs.

The linear regression of log $t_{1/2\beta}$, log$V_{d(area)}$, and log Cl_B versus body weight was analyzed so that intercept *a* and slope *b* could be computed following the equation

$$LogY = c + b \,.\, logW$$

After that the allometric equation could be applied to

$$Y = a \,.\, W^b$$

where Y is the value of the corresponding pharmacokinetic parameter ($t_{1/2\beta}$, $V_{d(area)}$ and Cl_B), a is the antilogarithm of c and W is the body weight.

The least square linear regression method was used for estimation of the correlation between pharmacokinetic parameters and body weight. Data were compared on the basis of Levenberg-Marquardt algorithm.

Statistical analysis

The Statistica 6.1 (Statistica for Windows; StatSoft Inc., USA) software was used. Pharmacokinetic parameters were presented as mean ± standard deviation (SD).

RESULTS

Serum spectinomycin concentrations, obtained by the microbiological method, were detected up to 4 h after *i.v.* administration. Spectinomycin concentrations measured by HPLC were detected up to 12 h after medication **(Table 1)**.

Table 1. *Serum concentrations of spectinomycin (mean±SD) after i.v. administration in goats (n=6) at a dose of 20 mg/kg body weight.*

Time (h)	Microbiological method (µg/mL)	HPLC method (µg/mL)
0.083	167.69±19.76	73.81±21.7
0.25	91.46±27.98	53.82±10.55
0.5	68.68±26.87	39.62±6.72
1	38.03±15.04	26.46±3.55
2	18.95±10.55	15.84±3.03
3	11.06±3.42	8.05±2.68
4	8.49±0	4.96±1.53
6	-	2.24±1.04
8	-	0.99±0.47
10	-	0.52±0.19
12	-	0.27±0.15

Antibiotic concentrations assayed microbiologically were higher than those measured by HPLC (**Figures 1, 2**).

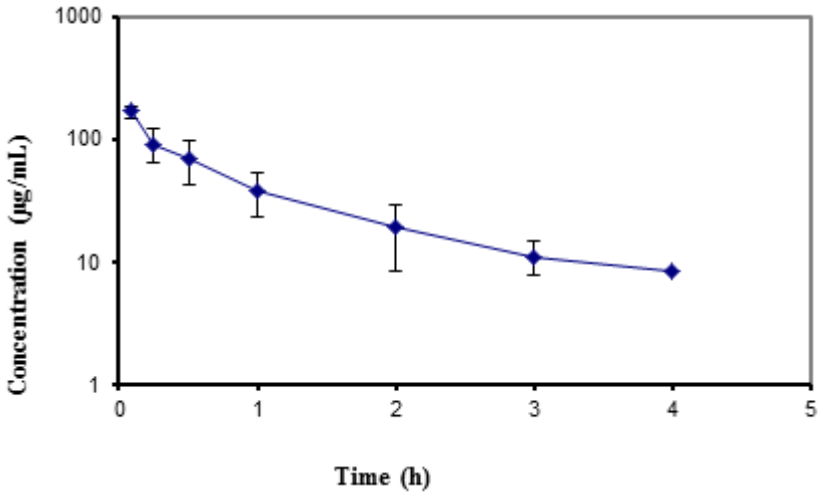

Figure 1. Serum concentrations of spectinomycin (mean ± SD) in goats after single intravenous administration at 20 mg/kg body weight determined by microbiological assay

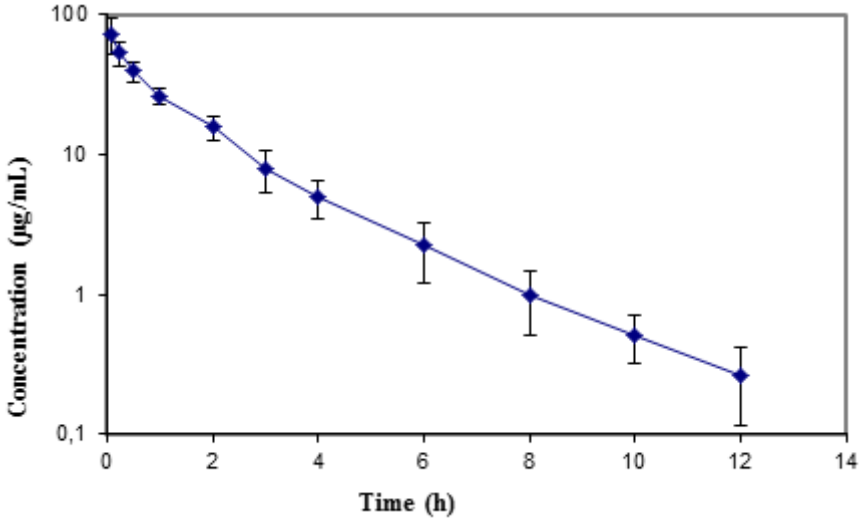

Figure 2. Serum concentrations of spectinomycin (mean ± SD) in goats after single intravenous administration at 20 mg/kg body weight determined by HPLC.

Blood serum concentrations of spectinomycin versus time curves fitted the following biexponential functions:

Following microbiological method:
Ct=185.11.exp(-28.2811.t)+93.5.exp(0.8398.t)

Following HPLC: ***Ct = 86.35.exp(-5.6433.t) + 31.39.exp(-0.4311.t)***

Selected pharmacokinetic parameters, calculated on the basis of a two-compartmental model are presented in **Table 2**.

Table 2. *Pharmacokinetic parameters of spectinomycin after single i.v. administration at a dose of 20 mg/kg body weight to goats (n = 6). The results were compared on the basis of microbiological and HPLC methods.*

Pharmacokinetic parameters (units)	Microbiological method	HPLC method
C^0 (μg/mL)	278.6±89.05	117.74±95.97
$t_{1/2\alpha}$ (h)	0.05±0.05	0.33±0.27
$t_{1/2\beta}$ (h)	0.8±0.17	1.74±0.51
$AUC_{0-\infty}$ (h.μg/mL)	171.67±64.03	96.26±12.62
Cl_B (mL/kg/min)	2.174±0.798	3.512±0.448
MRT (h)	0.89±0.25	1.87±0.428
V_{ss} (L/kg)	0.117±0.06	0.391±0.09
$V_{d(area)}$ (L/kg)	0.147±0.051	0.527±0.158
k_{10} (h^{-1})	9.5129±12.392	1.1941±0.8493
k_{12} (h^{-1})	14.7687±13.05	2.9225±4.3343
k_{21} (h^{-1})	2.7431±1.0227	1.9578±1.5411

Data were presented as mean ± SD.
C^0 – initial serum concentration; $t_{1/2\alpha}$ – distribution half-life; $t_{1/2\beta}$ – elimination half-life; $AUC_{0-\infty}$ – area under the plasma concentration-time curves from zero to infinity; Cl_B – total body clearance; MRT – mean residence time; V_{ss} – steady-state volume of distribution; $V_{d(area)}$ – area volume of distribution; k_{10} – elimination rate constant from central compartment; k_{12} – distribution rate constant from central to peripheral compartment; k_{21} – distribution rate constant from peripheral to central compartment.

Some basic values of pharmacokinetic parameters after *i.v.* administration of spectinomycin, submitted by various authors, are presented in **Table 3**. On the basis of them are calculated the allometric equations (**Table 4**).

Table 3. *Values of some pharmacokinetic parameters after i.v. administration of spectinomycin*

Species	Body weight (kg)	$t_{1/2\beta}$ (h)	$V_{d(area)}$ (L)	Cl_B (mL/min)	Authors
Cattle	528	1.01	158.4	1811.04	Ziv & Sulman (1973)
Sheep	56.5	1.1	17.52	184.19	Ziv & Sulman (1973)
Sheep	35.5	2.078	–	46.15	Radi (2012)
Goat	45.5	1.74	23.98	159.77	Our data
Goat	45.5	0.8	6.689	98.92	Our data
Chicken	2.0	1.46	0.68	5.36	Abu-Basha *et al.* (2007)
Rat	0.2	0.754	0.149	54.08	Madhura *et al.* (2013)

$t_{1/2\beta}$ – elimination half-life; $V_{d(area)}$ – volume of distribution; Cl_B – total body clearance.

Table 4. *Values of allometric parameters for spectinomycin*

Equation	**n**	**r**	**p**
$t_{1/2\beta} = 1.19.W^{0.02}$	7	0.1619	NS
$V_{d(area)} = 0.37.W^{0.96}$	6	0.9962	<0.001
$Cl_B = 1.92.W^{1.09}$	7	0.9985	<0.001

$t_{1/2\beta}$ – elimination half-life; $V_{d(area)}$ – volume of distribution; Cl_B – total body clearance; n – number of the studied species; r – correlation coefficient; p – level of significance of correlation coefficient.

DISCUSSION

The purpose of the present study was to describe the pharmacokinetics of spectinomycin, which has not been studied in goats yet. To accomplish that two methods for measuring serum concentrations were used and compared – microbiological and HPLC. Because of the lack of metabolites, high sensitivity and cost-effectiveness the microbiological assay is sometimes considered

better for assessment of aminoglycoside concentrations in different bodily fluids (10). However, in this study HPLC showed significantly higher sensitivity than microbiological assay. The LOQ in microbiological method was 6.25 μg/mL, but in HPLC assay it was 0.1 μg/mL. Because of that serum concentrations, obtained by the microbiological method were detected up to 4th h after *i.v.* administration and by HPLC - up to 12th h. This caused significant differences in the calculated pharmacokinetic parameters. The values of $t_{1/2\beta}$, Cl_B and $V_{d(area)}$ computed on the basis of the serum concentrations obtained by HPLC were considerably higher than the corresponding values calculated by microbiologically determined serum concentrations. The opposite is true regarding the rate constants k_{10}, k_{12} and k_{21}. The data from validation of the method also showed the advantage of HPLC assay. All of this made HPLC the preferable method for spectinomycin determination because of its sensitivity and accuracy. The following discussion will make use of the pharmacokinetic parameters computed on the basis of HPLC-determined serum concentrations.

Our data of spectinomycin pharmacokinetics in goats are generally similar to the corresponding data describing the kinetic behavior of different aminoglycosides (gentamicin, amikacin, tobramycin, kanamycin and apramycin) in goats (11). Spectinomycin was distributed more slowly in goats ($t_{1/2\alpha}$ = 0.33 h) than in sheep (0.175 h) and rats (0.237 h) (12, 13). Considering the high dose administered (20 mg/kg b.w.) the values for $AUC_{0-\infty}$ and C^0 were comparatively low - 96.258 h.μg/mL and 117.74 μg/mL, respectively. For example $AUC_{0-\infty}$ in sheep subjected to the same dose was 254.366 h.μg/mL and C^0 was 281.32 μg/mL (12). However C^0 values, obtained in this study were greater than the same published for cattle (67.2 μg/mL) and sheep (70.5 μg/mL) (14). The mean value of rate constant k_{12} in goats was higher than the mean value of k_{21}. This is one of the reasons for the high elimination rate of this aminocyclitol, signified by high Cl_B values.

The elimination half-life ($t_{1/2\beta}$) expresses the overall rate of drug elimination and the mean value obtained in our study (1.74 h) was similar to $t_{1/2\beta}$ reported in chicken (1.46 h) and sheep (2.078 h) (12, 15). The mean values following experiments with cattle (1.01 h) and sheep (1.1 h), however, were somewhat lower (14). After *i.v.* administration of goats with other aminoglycosides the values of $t_{1/2\beta}$ can be considered similar to ours (1.32 – 2.42 h) (11). The mean value of the total body clearance (Cl_B - 3.512 mL/kg/min) after spectinomycin medication of goats, however, is higher than the same values reported after administration of the other aminoglycosides (1.816 – 3.23 mL/kg/min) (11). The same is true regarding spectinomycin Cl_B in sheep (1.3 mL/kg/min) and chickens (2.68 mL/kg/min) (12, 15). The Cl_B in goats was similar to those reported in cattle (3.43 mL/kg/min) and sheep (3.26 mL/kg/min) (14).

The volume of distribution $V_{d(area)}$ (0.527 L/kg) would suggest that the distribution of spectinomycin in goats is limited mainly to the extracellular fluid. Nevertheless, this value is comparatively high considering the lower values of $V_{d(area)}$ in goats, administered with other aminoglicosides (11). The $V_{d(area)}$ values are higher than the respective values obtained after cattle (0.3 L/kg), sheep (0.31 L/kg) and chicken (0.34 L/kg) trials (14, 15). The steady-state volume of distribution V_{SS} (0.391 L/kg) is higher than those reported in sheep (0.187 L/kg) and chickens (0.26 L/kg) (12, 15).

Regarding the allometric equation for elimination half-life ($t_{1/2\beta} = 1.19.W^{0.02}$) the intercept value *a* in our study is within the range reported for other aminoglycosides (*a* = 0.77 - 1.53), but the slope value *b* is very low (8). Moreover, the half-life values are showing lack of correlation to body weight, most likely because of the different methods for spectinomycin determination and the relatively few species examined. Volume of distribution allometric equation ($V_{d(area)} = 0.37.W^{0.96}$) and total body clearance allometric equation ($Cl_B = 1.92.W^{1.09}$), however, have high level of correlation to body weight (<0.001). Moreover, calculated values of $V_{d(area)}$ and Cl_B on the basis of the allometric equation are very similar to those reported in a variety of allometric studies of different aminoglycosides (8, 16, 17) and therefore can be used.

CONCLUSIONS

Because of the higher sensitivity and accuracy HPLC assay can be considered as better choice for spectinomycin determination in comparison with microbiological method. Spectinomycin was distributed mainly in the extracellular fluid and the rate of elimination was comparatively high. The allometric analysis showed some usefulness, but future allometric studies which include more species are recommended.

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