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Comparative pharmacokinetics and PK/PD parameters of five aminoglycosides in goats

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Abstract: The pharmacokinetics of gentamicin, tobramycin, amikacin, kanamycin, and apramycin were investigated after single intravenous administration in goats. Their antimicrobial activity against 30 strains of *Staphylococcus aureus* isolates from goats was also studied. On the base of the pharmacokinetic and pharmacodynamic data, a comparison between antibiotics was made.

All 5 antibiotics showed a marked high in vitro antibacterial activity. The lowest MIC_{90} values were those of amikacin and the highest one was of kanamycin. The ratios of Cmax/MIC showed that amikacin has an advantage of providing the highest values of the concentration/MIC_{90} ratio, as well of the time providing bactericidal levels, followed by apramycin, whereas the other 3 antibiotics had comparable values, but lower than the first 2 antibiotics. The same interpretation could be made for the area under the curve/MIC_{90} ratio.

Key Words: Aminoglycosides, pharmacokinetics, antibacterial action, PK/PD, goats

Introduction

Aminoglycoside antibiotics are widely used in veterinary medicine, primarily for the treatment and prevention of gram-negative infections. The most common investigations in this area are on their pharmacodynamics and, particularly on their antimicrobial activity (1-4). The studies on their pharmacokinetics are also conformed to their activity against gram-negative agents (5-8). It is well known, however, that aminoglycosides exhibit an antibacterial activity against gram-positive microorganisms and are used for treatment of diseases caused by these organisms (9). Data based on the integration of pharmacokinetic parameters in different species and data for the antimicrobial activity of aminoglycosides against gram-negative microorganisms were reported (4,10-12), but with regard to gram-positive bacteria, they are limited or lacking. The number of publications presenting comparative pharmacokinetic and pharmacodynamic data for the members of this group is also scarce (3,13,14). There are no previous reports on comparative pharmacokinetics and

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pharmacodynamics of aminoglycoside antibiotics in goats. Therefore, the present study aimed to compare several basic pharmacokinetic parameters of 5 aminoglycosides and aminocyclitols (gentamicin, tobramycin, amikacin, apramycin, and kanamycin) in goats and the pharmacodynamic indices showing their activity against staphylococcal isolates from caprine.

Material and Methods

Animals

Experiments with each antibiotic were performed on a group of 6 adult (2-4 years) non-lactating, non-pregnant goats. During the time of the experiments, the animals weighed between 38.62 ± 3.51 and 45.9 ± 3.51 kg. They were fed standard grass and concentrate mixtures according to the requirements of the species and fasted 12 h prior to the respective experiment beginning. Drinking water was provided ad libitum.

Drugs

The medications used and their doses are presented in Table 1.

Experimental procedure

Antibiotics were injected once intravenously as a single bolus in *v. jugularis*. Blood samples from all treated animals were withdrawn prior to and at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post treatment from the contralateral vein. They were kept at room temperature for 2 h and the separated serum stored for 48 h at -20 °C until the microbiological assay (15). In Table 2 are presented the characteristics of the method for the antibiotics used.

Pharmacokinetic analysis

Pharmacokinetic parameters, based on the serum concentrations, were calculated with the WINNONLIN 4.0.1. (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA) software using the compartmental approach. The choice of a model of best fit to blood concentration curves was performed according to Akaike's criterion.

Statistical analysis

The values of pharmacokinetic parameters are presented as mean ± standard deviation (mean ± SD).

Table 1. Antibiotics used in the investigation.

Antibiotic	Drug form (company, city, country)	Drug concentration	Dose (mg/kg)
Amikacin	Ampoules amikacin sulphate 2 ml (Sopharma, Sofia, Bulgaria)	25%	10
Tobramycin	Substance tobramycin 983 μg/ml (Actavis, Sofia, Bulgaria)	10%	5
Apramycin	Phials apramycin sulfate 50 ml (Actavis, Sofia, Bulgaria)	20%	20
Gentamicin	Phials gentamicin sulfate 50 ml (Actavis, Sofia, Bulgaria)	10%	4
Kanamycin	Phials kanamycin sulfate 100 ml (Alfasan, Worden, Holland)	25%	10

Table 2. Characteristics of the antibiotic determination methods using *Bacillus subtilis* ATCC 6633.

Antibiotic	LOQ (mg/ml)	r^2	Intra-assay CV (%)	Inter-assay CV (%)
Amikacin	0.08	0.9892	7.12	14.38
Tobramycin	0.02	0.9949	5.23	8.62
Gentamicin	0.02	0.9802	3.43	9.76
Kanamycin	0.16	0.9858	5.14	6.37
Apramycin	0.04	0.995	7.34	11.42

Microbiological studies

The study was performed with a total of 30 strains belonging to *Staphylococcus* spp. Out of them, 19 were identified as *Staphylococcus aureus* and 11 as coagulase-negative species, identified as *Staphylococcus epidermidis* (7 strains), *Staphylococcus caprae* (2 strains), and *Staphylococcus xylosus* (2 strains). They were isolated from goats suffering from various local infections.

The isolates were identified by Gram staining; haemolytic activity, tests of catalase, oxidase and motility, fermentation of mannitol, production of DNA-ase and plasmocoagulase as well as additional identification were carried out by the semi-automated system CRYSTAL (Becton Dickinson).

For MIC determination, the macrobroth dilution test was performed in tubes with a final volume of 1 ml according to "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically". Approved Standard M7-A3. Clinical and Laboratory Standard Institute, Villanova, Pa. 1999 (16).

For quality control of the method, reference strains providing MIC values to all tested antimicrobials according to CLSI requirements were selected. In concordance to the CLSI method, MIC was interpreted using *Staphylococcus aureus* ATCC 25922 as reference strain. The theoretical curves, showing the relationship between the number of sensitive strains and antibiotic concentrations, were used to obtain MIC_{90}.

On the basis of selected pharmacokinetic parameters and MIC_{90} values of the 5 antibiotics, several PK/PD parameters were calculated.

Results

Pharmacokinetics

The blood serum concentration versus time curves of the 5 antibiotics (Table 3) fitted biexponential functions:

Gentamicin: Ct = 24.19.exp(-4.1731.t) + 11.42.exp(-0.4349.t);

Amikacin: Ct = 77.23.exp(-4.3614.t) + 23.37.exp(-0.337.t);

Tobramycin: Ct = 29.67.exp(-9.714.t) + 8.66.exp(-0.4029.t);

Kanamycin: Ct = 51.15.exp(-6.3768.t) + 20.01.exp(-0.3376.t);

Apramycin: Ct = 174.66.exp(-7.6728.t) + 53.47.exp(-0.528.t).

Selected pharmacokinetic parameters, calculated based on a 2-compartment model, are presented in Table 4.

Table 3. Serum concentrations of gentamicin, tobramycin, amikacin, kanamycin, and apramycin after single i.v. administration in goats (mean ± SD).

Time (h)	Gentamicin (4 mg/kg)	Amikacin (10 mg/kg)	Tobramycin (5 mg/kg)	Kanamycin (10 mg/kg)	Apramycin (20 mg/kg)
0.083	28.0 ± 4.46	76.77 ± 9.79	21.44 ± 8.41	47.38 ± 3.65	140.29 ± 25.03
0.25	20.05 ± 3.8	51.27 ± 6.88	13.96 ± 6.74	25.43 ± 4.84	70.62 ± 8.4
0.5	12.55 ± 1.3	37.59 ± 5.64	10.18 ± 3.77	22.66 ± 5.77	45.86 ± 8.83
1	7.88 ± 1.27	17.96 ± 3.63	6.75 ± 1.96	14.84 ± 3.3	31.28 ± 5.26
2	5.77 ± 1.15	11.02 ± 3.56	3.51 ± 0.42	10.69 ± 1.21	17.36 ± 2.64
3	1.94 ± 0.33	10.55 ± 3.60	2.54 ± 0.53	9.15 ± 2.05	12.99 ± 2.4
4	1.57 ± 0.25	6.84 ± 1.64	1.79 ± 0.23	4.38 ± 0.53	5.77 ± 2.6
6	1.28 ± 0.29	3.69 ± 1.94	0.88 ± 0.15	2.35 ± 0.38	2.12 ± 1.73
8	0.84 ± 0.46	1.63 ± 0.69	0.52 ± 0.12	1.28 ± 0.26	0.5 ± 0.31
10	0.44 ± 0.24	0.74 ± 0.3	0.34 ± 0.08	0.48 ± 0.12	0.21 ± 0.24
12	0.23 ± 0.13	0.47 ± 0.15	0.22 ± 0.06	0.28 ± 0.11	0.05 ± 0
24	0.02 ± 0.01				

Table 4. Pharmacokinetic parameters of aminoglycosides in goats (mean ±SD).

Parameters	Units	Antibiotics				
		Gentamicin	Amikacin	Tobramycin	Kanamycin	Apramycin
Dose	mg/kg	4	10	5	10	20
AUC	h.μg/ml	33.09 ± 5.13	95.13 ± 20.58	26.75 ± 5.65	69.61 ± 8.6	123.4 ± 18.04
AUC/D		8.27 ± 1.28	9.51 ± 2.06	5.351 ± 1.13	6.96 ± 0.86	6.17 ± 0.9
$t_{1/2a}$	h	0.19 ± 0.07	0.23 ± 0.12	0.17 ± 0.13	0.18 ± 0.1218	0.1±0.04
$t_{1/2b}$	h	1.81 ± 0.85	2.42 ± 0.8	1.82 ± 0.5	2.06 ± 0.5884	1.32 ± 0.09
C^0	μg/ml	35.6 ± 7.2	100.6 ± 23.4	38.3 ± 18.3	71.2 ± 24.8	228.1 ± 65.9
Cl_B	ml/min/kg	2.06 ± 0.347	1.816 ± 0.36	3.23 ± 0.67	2.42 ± 0.31	2.759 ± 0.479
AUMC	h^2.μg/ml	71.51 ± 36.39	259.80 ± 128.47	57.23 ± 15.3	180.99 ± 46.0	195.15 ± 35.1
MRT	h	2.1 ± 0.81	2.6 ± 0.81	2.17 ± 0.5	2.59 ± 0.88	1.58 ± 0.08
Vss	l/kg	0.252 ± 0.073	0.270 ± 0.054	0.428 ± 0.16	0.375 ± 0.177	0.26 ± 0.038
K_{12}	h^{-1}	1.614 ± 1.267	2.138 ± 1.643	6.448 ± 8.39	3.844 ± 3.327	4.124 ± 1.432
K_{21}	h^{-1}	1.584 ± 1.108	1.4293 ± 1.24	2.190 ± 1.29	1.821 ± 0.78	2.215 ± 0.487
$Vd_{(area)}$	l/kg	0.312 ± 0.114	0.366 ± 0.11	0.508 ± 0.16	0.431 ± 0.186	0.315 ± 0.059
$\beta.100/K_{10}$	%	40.45 ± 12.2	29.93 ± 7.9	32.42 ± 13.3	36.44 ± 14.39	30.86 ± 11.17

D – dose;
$t_{1/2}$ - distribution half-life;
$t_{1/2}$ - elimination half-life;
AUC - area under the concentration-time curves;
V_{darea}, - volume of distribution;
Vss - steady-state volume of distribution;
MRT - mean residence time;
Cl_B - total body clearance;
AUMC - area under the first moment curve;
MRT – mean residence time

Minimum inhibitory concentrations

The observed MIC values for gentamicin are within the range of 0.2 - 0.5 μg/ml. There was no intermediate or resistant isolate. MIC_{90} was 0.5 μg/ml (Figure).

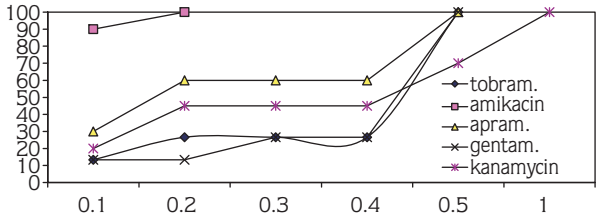

Figure. Curves of the antibacterial activity of tobramycin, amikacin, apramycin, gentamicin, and kanamycin, presented through MIC, against caprine *Staphylococcus* spp. isolates.

Amikacin. MIC values ranged between 0.1 and 0.2 μg/ml. MIC_{90} was 0.1 μg/ml, the lowest inhibitory concentration determined for all tested antibiotics.

All 30 tested *Staphylococcus* spp. strains showed a marked sensitivity to tobramycin. The determined inhibitory concentrations were from 0.1 to 0.5 μg/ml, MIC_{90} was 0.5 μg/ml.

For kanamycin, the MIC range was between 0.1 and 1.0 μg/ml. MIC_{90} was 1 μg/ml; or kanamycin showed the lowest activity, compared to the tested aminoglycosides and apramycin.

MIC values for apramycin ranged between 0.1 and 0.5 μg/ml, i.e. all 30 tested isolates showed a marked sensitivity. MIC_{90} was 0.5 μg/ml - equal to those of tobramycin and gentamicin.

The values of PK/PD calculations of the 5 antibiotics are presented in Table 5.

Discussion

In the present paper the pharmacokinetic data were calculated on the base of recommended and used doses. The serum antibiotic levels and the respective pharmacokinetic parameters allowed a

Table 5. Ratios of some pharmacokinetic parameters of aminoglycosides in goats to MIC_{90} against staphylococci (mean ± SE).

Antibiotic	Parameters			
	$C°/MIC_{90}$	$C_{(5\ min)}/MIC_{90}$	$T\ (C_{10}) > MIC_{90}$ (h)	AUC/MIC_{90}
Gentamicin	71.22 ± 5.89	56 ± 3.64	2.08 ± 0.11	73.07 ± 4.31
Amikacin	998.43 ± 97.93	767.68 ± 39.97	9.22 ± 0.42	969.67 ± 66.4
Tobramycin	76.65 ± 14.98	42.87 ± 6.87	1.38 ± 0.13	56.1 ± 5.3
Kanamycin	71.16 ± 10.33	47.38 ± 1.63	2.44 ± 0.38	68.86 ± 3.45
Apramycin	456.27 ± 53.84	280.57 ± 20.44	4.4 ± 0.39	250.98 ± 15.96

$C°/MIC_{90}$ – ratio of C° to MIC_{90};
$C_{(5\ min)}/MIC_{90}$ – ratio of the concentration by post injection min 5 to MIC_{90};
$T\ (C_{10}) > MIC_{90}$ – time that plasma concentration remains higher than 10 times the MIC_{90};
AUC/MIC_{90} – ratio of area under the curve (AUC) to MIC_{90}.
$T(C_{10})$ – time that plasma concentrations remain higher than 10 times the MIC_{90}

general evaluation that they present the typical pharmacokinetics of aminoglycosides – a short distribution phase and low volume of distribution, as well as persistence within detectable concentration range in at least 12 h. In the available literature, there is a varying number of data about gentamicin, amikacin, apramycin, and kanamycin pharmacokinetics in goats (17-22). Our data confirmed at a considerable extent the results of other authors. At the same time, however, the significant variations in the data of various investigators about the different antibiotics should also be taken into consideration (23,24).

Despite the observed similar values describing the pharmacokinetics of the 5 examined antibiotics, in the present paper some variations could also be distinguished. The distribution of apramycin was the fastest whereas that of amikacin was the slowest. According to the distribution volumes data, the antibiotics were arranged in descending order as followed: tobramycin, kanamycin, amikacin, apramycin, and gentamicin. According to the percentage of antibiotic in the central compartment after the finished distribution, the order was: amikacin, apramycin, tobramycin, kanamycin, and gentamicin. Although the differences in the volumes of distribution may have species-related specificity in guinea pigs, the distributions of gentamicin and tobramycin are reported to be the same (13). There is also a well-defined difference in the intercompartmental transfer of examined antibiotics. The most intensive transfer was that of tobramycin, followed by apramycin, and the least intensive one was that of gentamicin. A more intensive transfer of tobramycin compared to gentamicin was established also in guinea pigs (14).

The elimination rate of antibiotics also exhibited differences. The fastest elimination rate was that of apramycin, followed by gentamicin, tobramycin, kanamycin, and amikacin. With regard to their bactericidal concentration-depending effect, the time of persistence was not of primary significance.

All 5 antibiotics showed a marked high in vitro antibacterial activity. Concentration-depending killing is a characteristic of aminoglycosides. The major pharmacodynamic parameters that correlate with clinical and bacteriologic efficacy of these drugs are 24-h area under serum drug concentration curve (AUC) to MIC ratio (≥25-30), or the peak drug concentration to MIC ratio (≥10-12) based on unbound serum concentration values (25). The bound part of the serum levels of aminoglycosides are very low and can be ignored.

The ratios between the investigated parameters to MIC_{90} showed that amikacin has an advantage of providing the highest values of the concentration/MIC_{90} ratio as well of the time of

providing bactericidal or inhibitory levels, followed by apramycin, whereas the other 3 antibiotics had comparable values, but lower than the first 2 antibiotics. The same interpretation could be made for the area under the curve/MIC_{90} ratio.

Our data allow us to expect some advantages of amikacin followed by apramycin as active antibiotics against *Staphylococcus aureus* strains compared to other aminoglycosides (tobramycin, gentamicin, and kanamycin).

References

1. Dhawan, V., Marso, E., Martin, W. J., Young, L.S.: In vitro studies with netilmicin compared with amikacin, gentamicin, and tobramycin. Antimicrob. Agents Chemother., 1977; 11: 64-73.
2. Freidlin, P.J., Bock, R., Bernath, A., Hoexter, S., Ziv, G., Fromer, A., Perlstein, Z., Tomer, S., Samberg, Y.; Apramycin: minimal inhibitory concentrations for avian *Esherichia coli* and serum levels after intramuscular injection in turkeys. J. Vet. Pharmacol. Ther., 1985; 8: 105-109.
3. Yoshimura, H., Ishimaru, M., Kojima, A.: Minimum inhibitory concentrations of 20 antimicrobial agents against *Staphylococcus aureus* isolated from bovine intramammary infections in Japan. J. Vet. Med. B, Infect. Dis. Vet. Public Health, 2002; 49: 457-460.
4. Müller, M., dela Peña, A., Derendorf, H.: Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. Antimicrob. Agents Chemother., 2004; 48: 1441-1453.
5. Cox, H.U.: In vitro antimicrobial susceptibility of Salmonellae from animals in Louisiana. Am. J. Vet. Res., 1980; 41: 809-811.
6. Cox, H.U., Luther, D.G., Newman, S.S., Roy, A.F.: Comparison of antibiograms determined by disk diffusion and microdilution methods for selected gram-negative bacilli. Am. J. Vet. Res., 1981; 42: 546-551.
7. Cid, D., Píriz, S., Ruiz-Santa-Quiteria, J.A., Valle, J., Vadillo, S., dela Fuente, R.: In vitro susceptibility of *Escherichia coli* strains isolated from diarrhoeic lambs and goat kids to 14 antimicrobial agents. J. Vet. Pharmacol. Ther., 1996; 19: 397-401.
8. White, D.G., Ayers, S., Maurer, J.J., Thayer, S.G., Hofacre, C.: Antimicrobial susceptibilities of *Staphylococcus aureus* isolated from commercial broilers in northeastern Georgia. Avian Dis., 2003; 47: 203-210.
9. Phillips, W.E., Williams, B.J.: Antimicrobial susceptibility patterns of canine *Staphylococcus intermedius* isolates from veterinary clinical specimens. Am. J. Vet. Res., 1984; 45: 2376-2379.
10. Toutain, P.L., del Castillo, J.R., Bousquet-Mélou, A.: The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. Res. Vet. Sci., 2002; 73: 105-114.
11. Haritova, A.M., Djeneva, H.A., Lashev, L.D., Sotirova., P.G., Gurov, B.I., Dyankov, V.N.: Pharmacokinetics of gentamicin and apramycin in turkeys, roosters and hens in the context of pharmacokinetic-pharmacodynamic relationships. J. Vet. Pharmacol. Ther., 2004; 27: 381-384.
12. Mouton, J.W., Vinks, A.A.: Pharmacokinetic/pharmacodynamic modeling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. Clin. Pharmacokinet, 2005; 44: 201-210
13. Carbon, C., Contrepois, A., Lamotte-Barrillon, S.: Comparative distribution of gentamicin, tobramycin, sisomicin, netilmicin, and amikacin in interstitial fluid in rabbits. Antimicrob. Agents Chemother., 1978; 13: 368-372.
14. Chung, M., Parravicini, L., Assael, B.M., Cavanna, G., Radwanski, E., Symchowicz, S.: Comparative pharmacokinetics of aminoglycoside antibiotics in guinea pigs. Antimicrob. Agents Chemother., 1982; 22: 1017-1021.
15. Arret, B., Johnson, D.P., Kirshbaum, A.: Outline of details for microbiological assays of antibiotics: second revision. J. Pharm. Sci., 1971; 60: 1689-1694.
16. CLSI: Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically. Approved Standard M7-A3. Clinical Laboratory Standards Institute, Villanova, Pa. 1999.
17. Brown, S.A., Riviere, J.E.: Comparative pharmacokinetics of aminoglycoside antibiotics. J. Vet. Pharmacol. Ther., 1991; 14: 1-35.
18. Uppal, R.P., Verma, S.P., Roy, R.K., Garg, S.K.: Pharmacokinetics of amikacin sulphate in goats. Indian J. Pharmacol., 1992; 24: 123-125.
19. Garg, S.K., Verma, S.P., Uppal, R.P.: Pharmacokinetics of gentamicin following single-dose parenteral administration to goats. Br. Vet. J., 1995; 151: 453-458
20. Abo El-Sooud, K.: Pharmacokinetics of amikacin in lactating goats. J. Vet. Med. A, 1999; 46: 239-246.
21. Agrawal, A.K., Singh, S.D., Jayachandran, C.: Effect of fever on pharmacokinetics and dosage regimen of intramuscularly administered amikacin in goats. J. Vet. Sci., 2001; 2: 91-96.
22. Agrawal, A.K., Singh, S.D., Jayachandran, C.: Comparative pharmacokinetics and dosage regimen of amikacin in afebrile and febrile goats. Indian J. Pharmacol., 2002; 34: 356-360.
23. Lashev, L.D., Pashov, D.A., Marinkov, T.N.: Interspecies differences in the pharmacokinetics of kanamycin and apramycin. Vet. Res. Commun., 1992; 16: 293-300.
24. Ziv, G., Kurtz, B., Risenberg, R., Glickman, A.: Serum and milk concentrations of apramycin in lactating cows, ewes and goats. J. Vet. Pharmacol. Ther., 1995; 18: 346-351.
25. Jacobs, M.R.: Optimisation of antimicrobial therapy using pharmacokinetic and pharmacodynamic parameters. Clin. Microbiol. Infec., 2001; 7: 589-596.