Comparative Clinical Pharmacology of Gentamicin and Tobramycin in Neonatal Calves

G. Ziv and M. Storper
Ministry of Agriculture, Kimron Veterinary Institute, Bet Dagan, Israel.

M. Wanner
Federal research Station for Animal Production, Grangeneuve, Posieux, Switzerland.

J. Nicolet
Department of Veterinary Bacteriology, University of Bern, Bern, Switzerland.

Introduction

The response of gram-negative bacilliary infections in neonatal calves to antimicrobial therapy frequently has been unsatisfactory. Although the results of therapy may be determined by factors other than the therapeutic regimen employed, antimicrobial agents remain the cornerstone of therapy, and there has been a continued search of potentially effective new agents. Among the currently available agents, gentamicin provides the widest bactericidal spectrum against gram-negative bacilli. The clinical uses of gentamicin in veterinary medicine were reviewed recently by Clark (1977). This reviewer cited the case in which a group of 40 calves (1 to 3 weeks old) developed diarrhea caused by *Escherichia coli.* After the first affected calves failed to respond to chloramphenicol and sulphonamides, an intramuscular injection of 40 mg gentamicin was given every 12 hours for 3 to 4 days. This treatment was effective and it should be noticed that parenteral medication in this case was effective in controlling an intestinal infection. Recently, a related aminoglycoside, tobramycin, with antimicrobial spectra similar to gentamicin, have been developed and its potential clinical value in veterinary medicine has been briefly described (Clark, 1977a).

Very few reports are available on the pharmacology and clinical application of gentamicin in farm animals. Ziv & Sulman, 1974, Kormendy, 1977, Carli, Pradella, Pompa & Sonzogni, 1978) and, to the best of knowledge, none as yet on tobramycin. The purpose of the present study was to compare the *in vitro* activity of gentamicin and tobramycin on gram-negative bacteria isolated from clinical cases in cattle and to study blood levels of the two antibiotics in neonatal calves.

Materials and Methods

In vitro sensitivity tests

The sensitivity to gentamicin of 168 *E. coli,* 63 *Salmonella spp.*, 17 *Klebsiella pneumonia,* and 4 *Aerobacter aerogenes* isolated from cattle in Switzerland and the sensitivity to both gentamicin and tobramycin of 158 *E. coli,* 105 *Salmonella typhimutium,* 46 *Sal. dublin,* 26 *Salmonella spp.* and 32 *Speuodomonas aeruginosa* strains isolated from cattle in Israel was tested by the agar plate dilution method, using Mueller-Hinton agar. The inoculum consisted approximately 10^5 organisms, from an overnight broth cultures, which was applied on the surface of the agar surface using a multipoint inoculation device. Inoculated plates were incubated overnight at 37°C and the minimal inhibitory concentrations (MIC) of the antibiotics were determined from the lowest concentration of drug which completely inhibited growth.

Antibiotic treatments

Twelve Simmental calves (6 to 8 weeks old, and body weight 58 to 76 kg each) were used in trials involving gentamicin, and sixteen Israeli-Friesian calves (2 to 3 weeks old, weighing 48 to 52 kg each) were used in investigations involving tobramycin. The body weight of the calves were determined before each trial. The antibiotics (gentamicin sulphate, Schering Corp., U.S.A., lot GMC-5-M-7085, and Nebcin, brand of tobramycin, Eli Lilly, U.S.A. lot 78-3162-J) were administered by a deep intramuscular injection at the upper neck region. Gentamicin was injected to two groups of six calves each, one group at 1.5 mg/kg/day and the other at 3.0 mg/kg/day during three days. Tobramycin was injected once only to eight calves at 1.5 mg/kg and to the other eight calves at 3.0 mg/kg.

Blood sampling and antibiotic assay

Blood samples (jugular venipuncture) were collected 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours after treatment. Blood was allowed to clot at room temperature for 4 to 6 hours and the serum was separated by centrifugation and was kept at -

20°C pending assay. Aliquots of serum were obtained from the calves treated with gentamicin before the first treatment and after the third treatment and these were retained for blood urea determination. Ultrafiltrates of serum from the gentamicin and the tobramycin-treated calves, collected 2, 6, and 8 hours after the first treatment, were obtained by the method described previously (Ziv & Sulman, 1972).

Concentrations of antibiotics were determined by microbiological assay using *Bacillus subtilis* ATCC 6633 as test organism. Antibiotic assay paper discs, 6 mm in diameter, were used. The paper discs were dipped into serum samples and standard antibiotic solution prepared in antibiotic-free calf serum and were placed on the surface of Mueller-Hinton agar seeded with the test organism.

Kidney function tests

The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined twice in each calf treated with gentamicin, first at 1 to 3 days before treatment and again 5 to 7 days after the end of treatment, using the double isotope single-injection method (Wanner, Ziv, Nicolet, Noelpp & Roesler, 1980). Visual observations on the behavior of all the treated calves were made periodically for possible signs of neurological irritation.

Results

The MIC values are presented in Table 1. Very few differences in the values were found for a given bacterial species isolated either in Israel or Switzerland. With the exception of *Ps. aeruginosa*, more that 75% of the strains were inhibited as drug concentrations of 0.25 μg/ml and more than 95% of the strains were inhibited at concentrations less than 2 μg/ml. The data show that gentamicin and tobramycin have very similar activity against a given type of isolate.

Mean serum gentamicin concentrations for each of the three days of treatment are given in Table 2. After treatment at 1.5 mg gentamicin/kg the drug peaked in serum 1 hour lated at concentrations ranging between 3.7 and 3.95 μg/ml. The corresponding peaks for the 3.0 mg/kg doses were approximately twice as high as the peaks of the 1.5 mg/kg doses. Drug accumulation from day 1 to day.3 was not observed. Very great variations were seen in serum drug levels among the animals treated, and these are reflected in the large standard deviation values for each sampling period. These large variations were not due to any particular animal but were seen with almost every calf treated. Concentrations ≥2.5 μg/ml were maintained in serum during 10 hours after treatment with the 1.5 mg/kg dose and during 12 hours after the higher dose was applied. The elimination half-life of gentamicin was estimated to be 3 hours.

Mean serum tobramycin concentrations are presented in Table 3. After treatment with 1.5 mg tobramycin/kg the drug peaked in serum 30 minutes later at 4.68 μg/ml and the peak for the 3.0 mg/kg dose was 10.6 μg/ml. Within 1.5 to 2 hours, however, mean serum tobramycin concentrations were very similar to those seen after an equivalent dose of gentamicin was injected (Fig. 1), and the two drugs appear to leave serum at approximately the same rate. Compared to

Table 1

Minimal inhibitory concentrations of gentamicin and tobramycin for gram-negative bacteria isolated from clinical cases in cattle in Switzerland and Israel

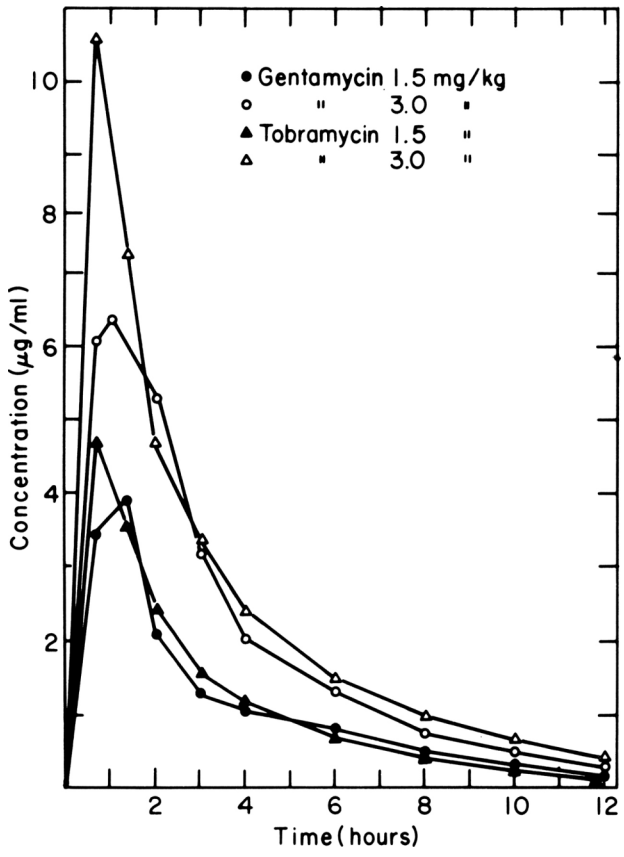
Bacteria	Anti-biotic	Country	No. of strains tested	Number of strains inhibited at given antibiotic concentration						
				0.125	0.25	0.5	1.0	2.0	4.0	8.0
	Genta	Switz	168		59	108	1			
Escherichia coli	Genta	Isr	158	12	86	34	20	2	2	2
	Tobra	Isr	158	12	87	33	21	3	2	
S. typhimurium	Genta	Isr	105		36	59	8	2		
	Tobra	Isr	105	4	32	60	7	2		
S. dublin	Genta	Isr	46	14	27	5				
	Tobra	Isr	46	16	25	5				
	Genta	Switz	63		41	22				
Salmonella spp.	Genta	Isr	26		19	7				
	Tobra	Isr	26	4	15	7				
K. pneumoniae	Genta	Switz	17			7	10			
Enterobacter aerogenes	Genta	Switz	4			4				
	Genta	Switz	9					8	1	
Ps. aeruginosa	Genta	Isr	32				4	26	2	
	Tobra	Isr	32				4	26	2	

Table 2

Serum gentamicin concentration in calves following intramuscular injections of gentamicin sulphate for three days at 1.5 mg/kg/day and 3.0 mg/kg/day

Hours after treatment	-------1.5 mg/kg/day------- First day Mean	S.D.	Second day Mean	S.D.	Third day Mean	S.D.	-------3.0 mg/kg/day------- First day Mean	S.D.	Second day Mean	S.D.	Third day Mean	S.D.
	concentration, ug/ml											
0.5	3.28	1.96	2.72	0.96	3.53	1.42	6.15	2.86	5.93	3.00	6.24	3.75
1	3.69	1.82	3.88	1.26	3.95	1.90	6.38	3.00	6.15	2.86	6.25	4.00
2	2.06	0.86	2.96	1.43	2.74	1.48	5.24	2.46	4.86	1.97	5.00	2.48
3	1.38	0.78	1.72	0.96	2.00	1.21	3.15	1.54	3.12	1.34	3.18	1.64
4	1.15	0.57	1.45	0.71	1.36	0.83	2.00	1.26	2.24	1.06	2.00	0.96
6	0.88	0.32	1.00	0.56	1.00	0.68	1.40	0.82	1.56	0.62	1.28	0.50
8	0.57	0.28	0.64	0.38	0.71	0.38	0.81	0.36	0.90	0.44	0.76	0.37
10	0.25	0.18	0.38	0.18	0.36	0.16	0.42	0.21	0.46	0.32	0.48	0.20
12	0.13	0.10	0.27	0.16	0.20	0.08	0.24	0.14	0.30	0.21	0.25	0.18
24	—		—		—		—		—		—	

Fig. 1. Mean serum gentamicin and tobramycin concentrations in calves after a single intramuscular injection of gentamicin at 1.5 mg/kg (n=6) and 3.0 mg/kg (n=6) and tobramycin at 1.5 mg/kg (n=8) and 3.0 mg/kg (n=8).



gentamicin, serum tobramycin concentrations at any given period after treatment were more uniform, calculated standard deviations were always less than those seen after treatment with gentamicin (Table 3).

The effect of intramuscular injection of gentamicin on the GFR and ERPF is shown in Table 4. In all but two calves (485 and 492) were the values for the GFR and ERPF higher after treatment than before treatment. In general, the magnitude of the increase in these values was reflected in the difference in body weight before and after the end of treatment.

Blood urea levels for the group of calves treated with gentamicin at 1.5 mg/kg/day were: before treatment -16.9 ± 4.5 mg/dl, and after treatment - 18.8 ± 4.5 mg/dl. For the calves treated at 3.0 mg/kg/day blood urea levels

Table 3

Serum tobramycin concentration in calves following intramuscular injection of tobramycin sulphate at 1.5 mg/kg and at 3.0 mg/kg

Hours after treatment	1.5 mg/kg Mean	S.D.	3.0 mg/kg Mean	S.D.
	concentration, ug/ml			
0.5	4.68	1.10	10.64	1.86
1	3.54	0.62	7.25	1.14
2	2.33	0.71	4.72	1.36
3	1.65	0.59	3.23	1.06
4	1.18	0.44	2.38	0.72
6	0.78	0.23	1.64	0.56
8	0.52	0.20	0.98	0.31
10	0.27	0.11	0.52	0.18
12	0.17	0.08	0.30	0.12
24	—		—	

Table 4

Effect of intramuscular injection of gentamicin once daily for three days at 1.5 and 3.0 mg/kg on the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) in calves

Calf No.	Dose (mg/kg)	GFR, ml/min/1.73 m²			ERPF, ml/min/1.73 m²			Percent body weight change
		Before treatment	After treatment	Percent change	Before treatment	After treatment	Percent change	
486	1.5	125	185	32.4	383	585	64.5	9.8
489	1.5	137	181	24.3	474	749	63.3	11.8
499	1.5	102	181	43.7	303	749	140.4	14.6
487	1.5	117	139	15.8	430	485	11.3	4.4
491	1.5	121	166	27.1	443	675	34.4	7.0
488	1.5	134	183	26.8	545	724	24.7	5.6
482	3.0	113	137	17.5	341	457	25.4	4.4
485	3.0	271	175	−54.9	873	864	−1.0	−1.5
492	3.0	142	141	−0.7	473	629	24.8	0.0
481	3.0	106	142	25.4	325	439	26.0	9.6
494	3.0	176	190	7.4	536	738	27.3	5.2
484	3.0	164	207	20.8	595	607	2.0	2.8

were: before treatment - 15.6 ± 3.3 mg/dl and after treatment 18.7 ± 5.5 mg/dl. Clinical observations did not reveal any change in general behavior of the animals which can be linked to neurological irritation.

Gentamicin and tobramycin were found to be bound to a very small degree in serum. In effect, the extent of binding (approximately 10%) is within the range of error inherent in the method (Ziv & Sulman, 1972).

Discussion

The antibiotic sensitivity patterns of the strains isolated from cattle were very similar to those reported for similar bacterial species of human origin (Hyam, Simberkoff Rahal, 1973, Karney, Holms & Turck, 1973) using the same method of sensitivity test. *Sal. typhimurim* is known to be more resistant than *Sal. dublin* to a variety of antibiotics and this difference apparently extends to gentamicin and tobtamycin too (Table 1). That differences were not found in the sensitivity patterns by strains isolated from cattle in Israel and Switzerland is to be expected as in both countries these drugs are not in use in farm animals. It was however, interesting to note the rather wide range of MIC values found for *E. coli* isolated in Israel. This finding may signal the possibility that resistant transfer factors may already be present in that country among a small proportion of the *E. coli* population of cattle origin.

Carli et al (1978) conducted a similar study with gentamicin in calves but reported that after a dose of 3.0 mg/kg/day peak gentamicin concentrations rose from 7.2 ± 1.1 μg/ml and rose to 8.3 ± 0.57 μg/ml on day 3. We have found slightly lower levels and this can perhaps be attributed to differences in the injection sites. The unpredictability of serum concentrations of gentamicin in man was reported by Kaye, Levison and Lebovitz (1974) who found that after a single intramuscular injection of gentamicin over a wide range of doses (0.83 to 3.14 mg/kg), peak levels in serum following the same dose vary widely and that rates of drug disappearance from serum vary considerably in patients with comparable renal function. Since gentamicin is eliminated by glomerular filtration only, similar to other aminoglycoside antibiotics, it could be logical to assume that the variability in serum drug levels is a reflection of differences in renal function among the calves. However, variations in renal function were not found (Table 4) neither before treatment nor after treatment. Although results from kidney function tests suggest that gentamicin does not produce renal empairment in calves when doses of 3.0 mg/kg are applied for three days, it is of interest to note that similar variations were not found after treatment with tobramycin.

The aminoglycosides are known to be distributed throughout the vascular and extracellular body compartment only. The apparent distribution volume of gentamicin in sheep was estimated to be not more than 7% of body weight (Ziv & Sulman, 1974). This peculiar feature of gentamicin, and perhaps of tobramycin too, may have some clinical relevance, at least as far as dosage schedules are concerned. Although both gentamicin and tobramycin were found to be bound to calf serum to a very small degree, a relatively large dose is apparently needed to create the necessary gradient of drug concentrations which enable drug molecules to reach infection sites outside the systemic circulation. The frequency of gentamicin's administration is

based partly on the duratin of therapeutic blood levels, i.e. near or above the MIC for the majority of pathogens, and partly on the seriousness of illness. If, for example, a calf were given 1.5 mg/kg of gentamicin once daily, the animal would have a bactericidal blood level for up to 10 hours and inadequate blood levels for the remaining 14 hours. Because this antibiotic is bactericidal, it would destroy most of the bacteria. The remaining bacteria would begin to grow, only to be destroyed by the next dose of gentamicin or the animal's immunity mechanism. The periods with less than therapeutic blood levels may be long enough, however, for growth of partially resistant organisms along with the transfer of R factors and subsequent failure of therapy (Clark, 1977). The greater effectiveness of higher blood levels, increased effectiveness of multiple daily doses, cost of the drug and its administration, and the seriousness of the disease have to be considered to obtain optimal results.

Summary

The minimal inhibitory concentrations (MIC) of gentamicin, and a relatively new closely related aminoglycoside - tobramycin, for gram-negative bacteria isolated from cattle was determined to be 0.25 μg/ml for at least 75% of the strains tested and 2.0 μg/ml for more than 95% of the isolates. Intramuscular injection of gentamicin at 1.5 mg/kg/day and 3.0 mg/kg/day for three days did not alter normal renal function. Both antibiotics were not bound to serum proteins. When administered at 1.5 mg/kg, both antibiotics produced serum levels in excess of the MIC during 10 hours and after the 3.0 mg/kg dose was injected effective blood levels were maintained during 12 hours. Gentamicin blood levels were rather erratic and unpredictable in the majority of animals treated. This peculiar behavior was not observed with tobramycin. Results are discussed in relation to optimal dosage schedules.

Acknowledgments

This study was supported in part by a grant given to G.Z. from the "Roache Research Foundation for Scientific Exchange and Biomedical Collaboration with Switzerland". We are grateful to the staff members of the Federal Research Station for Animal Production, Grangeneuve, Posieux for their cooperation.

References

--Carli, S., Pradella, G., Pompa, G. and Sonzogni, 0: La gentamincina new vitelli neonati: Faracocinetica, distribuzone tissutale, residui e tollerabilita. Arch. Vet. Ital.*29*, 64-68 (1978). — --Clark, C.H.: Clinical uses of gentamicin. Modern Vet. Pract. *58*, 751-754 (Sept. 1977). — --Clark, C.H.: Bacterial sensitivity to the aminoglycoside antibiotics. Modern Vet. Pract. (July 1977a). *58*, 675-678. – --Hyams, P.J., Simberkoff, M.S. and Rahal, J.J.: In vitro bactericidal effectiveness of four aminoglycoside antibiotics. Antimicrob. Agents Chemother. *3*, 87-94 (1973). — --Karney, W., Holms, K.K. and Turck, M.: Comparison of five aminocyclitol antibiotics in vitro against *Enterobacteriacea* and *Pseudomonas*. Antimicrob Agents Chemother. *3*, 338-342 (1973). — --Kaye, D., Levison, M.E. and Labovitz, E.D.: The unpredictability of serum concentrations of gentamicin: Pharmacokinetics of gentamicin in patients with normal and abnormal renal function. J. infec. Dis. *130*, 153-154 (1974). — --Kormendy, B.: Therapeutic value of gentamicin in the treatment of mastitis. Magyar Allator. Lapja *13*, 131-133 (Feb. 1977). — --Wanner, M., Ziv, G., Nicolet, J., Noelpp, U.P. and Roesler, H.: Experiments with double isotope single injection method for determining glomerular filtration rate and effective renal plasma flow in veal calves. Res. Vet. Sci. *30*, 239-240 (1981). — --Ziv, G. and Sulman, F.G.: Distribution of aminoglacoside antibiotics in blood and milk. Res. Vet. Sci. *17*, 68-74 (1974). — --Ziv, G. and Sulman, F.G.: Binding of antibiotics to bovine and ovine serum. Antimicrob. Agents Chemother. *2*, 206-213 (1972).