Selection of an aminoglycoside antibiotic for administration to horses

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Summary

The serum concentrations of the aminoglycosides neomycin, kanamycin and streptomycin were determined after intravenous (iv) and intramuscular (im) administration. These values were then related to the minimum inhibitory concentrations (MIC) of a number of equine pathogenic bacteria to determine the duration of therapeutic serum concentrations of the aminoglycosides in the horse. Pharmacokinetic analysis of the data using neomycin as the example revealed a mean ($\pm$ sd) peak serum concentration of 23.2 ± 10.2 μ g/ml present at 30 mins, and at 8 h the serum concentration was 2.8 ± 0.8 μ g/ml. From the pharmacological analysis of concentration-time data it was shown that neomycin was very rapidly absorbed from the im injection site, with an absorption half-time of 0.16 ± 0.05 and was well absorbed (systemic availability was 73.7 ± 26.9 per cent). A peak tissue level, which represented 40 per cent of the amount of drug in the body, was obtained at 32 mins after injection of the drug. At 8 h, the fractions of the dose in the central and peripheral compartments of the model were 1.5 per cent and 2.5 per cent respectively, and 96 per cent was the cumulative amount eliminated up to that time. Based on the MIC values of the majority of isolates of *Corynebacterium equi*, and only a few isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Streptococcus equi*, one would expect a serum concentration of more than 2 μ g neomycin/ml up to 8 h following im dosage (10 mg/kg) to be therapeutically effective.

Introduction

THE determination of the susceptibility of an organism to a particular antimicrobial agent is usually the result of a calculation which considers the *in vitro* minimum inhibitory concentration (MIC) value for the organism and the serum concentration of antimicrobial agent which can be achieved and maintained in the patient. These calculations have seldom been made for organisms and antimicrobial agents in horses or other domestic animals.

The work reported here was undertaken to determine the serum concentrations of some aminoglycosides that can be achieved in the horse and the MICs of a number of therapeutic serum concentrations of the aminoglycosides in the horse.

The aminoglycosides are generally considered to have their primary antibacterial activity against aerobic Gram-negative bacilli. These antibiotics have little activity against anaerobic

microorganisms or facultative bacteria under anaerobic conditions and their action against Gram-positive bacteria is limited. However, because the aminoglycosides are commonly used in equine practice alone or in combination with penicillin for broad-spectrum cover, the MICs of a number of Gram-positive organisms have been included in the present study.

Materials and methods

MIC determinations

MICs required to inhibit growth of the test organism for 18 h at 37° C were measured in duplicate. Serial dilutions of the aminoglycosides streptomycin, kanamycin and neomycin were made in nutrient broth (brain heart infusion; Oxoid). The inoculum was one drop (0.025 ml) of an overnight culture of the test organism in 2 ml of nutrient broth. The MIC was interpreted as the lowest concentration of aminoglycoside which inhibited growth of the organism. MICs for each of the three aminoglycosides were performed against the following organisms: *Corynebacterium equi*, 17 strains; *Pseudomonas aeruginosa*, 12 strains; *Klebsiella pneumoniae*, four strains; *Escherichia coli*, 17 strains; *Salmonella typhimurium*, nine strains; *Hafnia alvei*, two strains; *Enterobacter cloaca*, two strains; *Proteus mirabilis*, three strains; *Staphylococcus aureus*, 11 strains; *Streptococcus equi*, six strains; *Strep zooepidemicus*, seven strains; *Strep equisimilis*, six strains.

Serum aminoglycoside determinations and pharmacokinetic analyses

The bioavailability and disposition kinetics of streptomycin, kanamycin and neomycin were determined in this study and have been described previously by Baggot, Love, Rose and Raus (1981). Single doses (10 mg/kg) of each aminoglycoside preparation were administered intravenously (iv) and intramuscularly (im) to six Standardbred horses. Blood samples were collected at fixed times after drug administration and concentrations of the antibiotics were measured in serum by the agar plate diffusion method of Bennett, Brodie, Benner and Kirby (1966).

The serum concentration-time data were analysed by least squares non-linear regression, using the Autoan computer program (Sedman and Wagner 1976). The experimental constants (A, B, α and β), together with the dose (10 mg/kg), were used to calculate the pharmacokinetic terms which

TABLE 1: Antibacterial spectrum of the aminoglycosides streptomycin, kanamycin and neomycin against common Gram-negative equine pathogens

	Total number of strains tested	MIC ($\mu\text{g/ml}$) of aminoglycosides tested					
		Streptomycin (Number of strains)		Kanamycin (Number of strains)		Neomycin (Number of strains)	
<i>Pseudomonas aeruginosa</i>	12	25.0 (6)		50 (3)		3.125 (2)	
		50.0 (2)		100 (4)		6.25 (2)	
		>200 (4)		>200 (6)		25 (2)	
						50 (1)	
						100 (1)	
						>200 (4)	
<i>Klebsiella pneumoniae</i>	4	>200 (4)		1.56 (1)		0.78 (2)	
				6.25 (1)		1.56 (1)	
				>200 (2)		>200 (1)	
<i>Escherichia coli</i>	17	1.56 (1)		1.56 (3)		0.78 (2)	
		3.125 (2)		3.125 (3)		1.56 (3)	
		6.25 (4)		6.25 (2)		3.125 (2)	
		100 (2)		12.5 (2)		6.25 (2)	
		200 (1)		50 (1)		12.5 (1)	
		>200 (7)		>200 (6)		100 (1)	
						>200 (6)	
<i>Salmonella typhimurium</i>	9	25 (1)		3.125 (1)		0.78 (3)	
		50 (1)		6.25 (1)		1.56 (2)	
		100 (2)		12.5 (2)		3.125 (3)	
		200 (1)		25 (3)		12.5 (1)	
		>200 (4)		50 (1)			
				100 (1)			
<i>Hafnia alvei</i> (<i>Enterobacter hafnia</i>)	2	50 (1)		3.125 (1)		1.56 (1)	
		>200 (1)		12.5 (1)		3.125 (1)	
<i>Enterobacter cloaca</i>		3.125 (1)		3.125 (1)		3.125 (1)	
		50 (1)		6.25 (1)		6.25 (3)	
				12.5 (2)			
<i>Proteus mirabilis</i>	3	25 (2)		6.25 (1)		12.5 (2)	
		>200 (1)		>200 (2)		>200 (1)	

MIC Minimum inhibitory concentration

describe disposition kinetics of each antibiotic. They were also used to calculate the individual rate constants (k_{12} , k_{21} and k_{el}) that are associated with the two-compartment open model (Baggot 1977). The individual rate constants, in turn, served as the input data to a computer program which provided curves showing the level of drug (as fraction of iv dose) in each compartment of the pharmacokinetic model and the amount eliminated up to 8 h after drug administration.

The extent of drug absorption from the im injection site was obtained by the method of corresponding areas, in which area under the im and iv curves (from 5 mins to 12 h) was calculated by the trapezoidal rule.

Results

The MICs for the equine pathogens tested are shown in Tables 1 and 2, and the bioavailability and disposition kinetics of streptomycin, kanamycin and neomycin in the horse are listed in Table 3. The serum concentration-time data for im administration of the three aminoglycosides are shown in Fig 1.

Using neomycin as the example, the following is illustrative of the use of the above data for the selection and dosage of an aminoglycoside antibiotic in the horse. Fig 2 shows the serum concentration-time curve for neomycin sulphate injection (VR Laboratories). After injection, a mean ($\pm$ sd) peak serum concentration of $23.2 \pm 10.2 \mu\text{g/ml}$ was present at 30 mins and at 8 h the mean serum concentration was $2.8 \pm 0.8 \mu\text{g/ml}$. Pharmacokinetic analysis of the serum concentration-time data showed that neomycin was very rapidly absorbed from the im injection site, with an absorption half-time of $0.16 \pm$

0.05 and was well absorbed (systemic availability was 73.7 ± 26.9 per cent). Repeated dosing at 8 h intervals did not change the absorption and disposition kinetics of the drug (Baggot *et al* 1981).

Computer generated curves showing the levels of neomycin in the central and peripheral compartments of the two-compartment pharmacokinetic model (which was used to describe the disposition kinetics of the drug) and the fraction of dose eliminated (presumably amount in the urine) are shown in Fig 3. A peak tissue level, which represented 40 per cent of the amount of drug in the body, was obtained at 32 mins after iv injection of the drug. At 8 h, the fractions of the dose in the central and peripheral compartments of the model were 1.5 per cent and 2.5 per cent, respectively; 96 per cent was the cumulative amount eliminated up to that time.

As has been pointed out previously (Weinstein 1975a), therapeutically effective blood concentrations require the drug to be maintained at 2 to 4 times the MIC of the organism. Based on the MIC values of the majority of isolates of *C equi*, and a few only isolates of *K pneumoniae*, *E coli*, *S typhimurium* and *S equi* (Tables 1 and 2), one would expect a serum concentration of more than $2 \mu\text{g}$ neomycin/ml up to 8 h following im dosage (10 mg/kg) to be therapeutically effective. The upper limit of the therapeutic serum concentration range must be a concentration that does not produce toxic effects.

Discussion

Since their isolation (streptomycin 1944, neomycin 1949 and kanamycin 1957) the aminoglycosides have been used, almost

TABLE 2: Antibacterial spectrum of the aminoglycosides streptomycin, kanamycin and neomycin against common Gram-positive equine pathogens

	Total number of strains tested	MIC (μ g/ml) of aminoglycosides tested					
		Streptomycin (Number of strains)		Kanamycin (Number of strains)		Neomycin (Number of strains)	
<i>Corynebacterium equi</i>	17	12.5	(10)	6.25	(1)	0.78	(13)
		25	(6)	12.5	(1)	6.25	(4)
		50	(1)	25	(1)		
				50	(14)		
<i>Staphylococcus aureus</i>	11	6.25	(1)	0.78	(1)	1.56	(1)
		25	(3)	6.25	(1)	3.125	(1)
		50	(3)	12.5	(3)	6.25	(2)
		100	(3)	25	(4)	12.5	(5)
		200	(1)	200	(2)	100	(1)
						200	(1)
<i>Streptococcus equi</i>	6	0.78	(1)	0.78	(2)	0.78	(2)
		12.5	(2)	1.56	(1)	1.56	(1)
		25	(3)	3.125	(2)	3.125	(1)
				12.5	(1)	6.25	(2)
<i>Streptococcus zooepidemicus</i>	7	12.5	(3)	0.78	(1)	3.125	(3)
		25	(2)	6.25	(3)	6.25	(2)
		50	(2)	12.5	(2)	12.5	(2)
				1.25	(1)		
<i>Streptococcus equisimilis</i>	6	3.125	(1)	0.78	(4)	1.56	(2)
		12.5	(2)	3.125	(1)	3.125	(3)
		25	(3)	25	(1)	6.25	(1)

MIC Minimum inhibitory concentration

TABLE 3: Bioavailability and disposition kinetics of some aminoglycoside antibiotics in horses (mean $\pm$ sd, n = 6)

	Streptomycin	Kanamycin	Neomycin
Intravenous administration:			
Half-life (h)	3.40 $\pm$ 0.42	1.80 $\pm$ 0.17	2.10 $\pm$ 0.97
Apparent volume of distribution (ml/kg)	231 $\pm$ 40	228 $\pm$ 25	232 $\pm$ 61
Body clearance (ml/h.kg)	47.5 $\pm$ 7.9	88.5 $\pm$ 11.3	82.6 $\pm$ 23.4
Fraction of dose (at 8 h):			
Central compartment (%)	2.5	1.5	1.5
Peripheral compartment (%)	11.5	2.0	2.5
Eliminated (%)	86.0	96.5	96.0
Intramuscular administration:			
Half-time absorption (h)	0.34 $\pm$ 0.15	0.32 $\pm$ 0.04	0.16 $\pm$ 0.05
Peak serum concentration (μ g/ml)	43.4 $\pm$ 21.4	35.8 $\pm$ 5.7	23.2 $\pm$ 10.2
Fraction absorbed (%)	83.1 $\pm$ 16.8	100	73.7 $\pm$ 26.9
Apparent half-life (h)	3.83 $\pm$ 0.30	2.66 $\pm$ 0.51	2.58 $\pm$ 0.69

exclusively in human medicine, to treat infections caused by Gram-negative bacteria.

Streptomycin has been used for many years in veterinary medicine. On most occasions it is used in combination with penicillin to give a broad spectrum of action when empirical therapy is instituted. As can be seen from Table 1, however, few of the common equine pathogens remain susceptible to this antibiotic. No species is predictably sensitive to streptomycin and sensitivity testing of isolates is mandatory before this antibiotic should be considered for therapy. Kanamycin has been used infrequently in veterinary medicine and veterinary preparations of kanamycin are not readily available. This has added significantly to the cost of this preparation and often prohibited its use.

Serious toxicity (ototoxicity and nephrotoxicity) is a major limitation to the usefulness of the aminoglycosides and the same spectrum of toxicity is shared by all members of the group (Sande and Mandell 1980). Neomycin has gained the reputation of causing such severe renal toxicity and ototoxicity when administered parenterally in man that most authors suggest limitation of its use to topical therapy (Sande and

Mendell 1980). Care is also required in oral administration of neomycin to individuals with renal insufficiency. In these cases, enough neomycin is absorbed to enable toxic concentrations to accumulate in the circulation (Weinstein 1975b).

Until the work of Baggot *et al* (1981), pharmacokinetic data for the aminoglycosides streptomycin, kanamycin and neomycin had not been available. This, plus the paucity of data on MICs for horse pathogens, has prevented determination of effective dosage regimens for these antibiotics in the horse. The term dosage regimen includes size of dose (mg/kg), interval between successive doses (h) and route of administration of the drug preparation.

Systemic administration of aminoglycosides is necessary to achieve therapeutically effective blood levels as there is little absorption when given orally (Sande and Mandell 1980). However, to avoid the initial excessively high serum concentrations which iv administration produces, it is desirable to administer aminoglycoside antibiotics im.

Based on the data presented, the im administration of the parenteral preparation of neomycin sulphate at a dose rate of 10 mg/kg and given at 8 h intervals would be expected to

Fig 1. Serum concentration-time curves for streptomycin (o), kanamycin (□), and neomycin (Δ) based on mean concentrations obtained in six horses following im administration of single doses (10 mg/kg) of the parenteral preparations

Fig 2. Neomycin concentrations in serum of horses following im injection of a single dose (10 mg/kg) of neomycin sulphate injection. Each point represents the mean concentration $\pm$ sd, $n = 6$

maintain serum concentrations within the therapeutic range for treatment of *C equi* infections. The decision to use this drug should take into account the susceptibility of *C equi* (measured *in vitro*).

Because renal excretion, specifically glomerular filtration, is the sole mechanism of elimination of neomycin, a reduction in renal function would decrease the elimination rate of the drug. Neonatal animals of the majority of species, including foals three to six weeks old, have a lower rate of glomerular

Fig 3. Computer-generated curves showing the level of neomycin (as fraction of iv dose) in the central and peripheral compartments of the two-compartment pharmacokinetic model and the amount eliminated over a period of 8 h. The curves were based on mean values of the individual rate constants that are associated with the model: $k_{12} = 0.0446/\text{min}$, $k_{21} = 0.0367/\text{min}$ and $k_{el} = 0.0158/\text{min}$. O represent the mean serum concentrations of neomycin following iv injection of a single dose (10 mg/kg)

filtration than adult animals. Should it be necessary to administer neomycin to a foal less than six to eight weeks old, a 12 h dosage interval would be indicated. The clinical effectiveness of neomycin in treating a systemic infection caused by susceptible microorganisms could depend on how early in the course of the infection treatment is initiated and on the overall duration of therapy.

As shown in this paper and also illustrated by Barton and Hughes (1980) most strains of *C equi* are sensitive to neomycin. The usefulness of neomycin for treatment of *C equi* infections should be considered by clinicians treating this condition. It is a much less expensive drug than gentamicin which is used widely in North America for treatment of this condition; it is not a drug extensively used in man. It is considered, along with some antimicrobial policies adopted in man, that gentamicin should be reserved for use against organisms where no other antimicrobial agent is effective.

Other than the aminoglycosides, all antimicrobial drugs to which *C equi* is sensitive are bacteriostatic. Other agents are therefore less suitable for severe systemic disease, especially in animals with already compromised immune systems, such as foals with *C equi* infection. However, the question of renal toxicity of neomycin (and gentamicin), especially to the developing nephron of the neonatal foal, has not been addressed, despite their successful use in practice. Before unqualified systemic use of these agents can be recommended, this situation must be investigated.

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ABSTRACT

Digestive system and diseases

Ulcerative duodenitis in foals

ACLAND, H. M., GUNSON, D. E. and GILLETTE, D. M. (1983) *Vet. Pathol.* **20**, 653-661.

THIS paper describes the clinical and pathological features of primary duodenitis in foals in its acute, necrotising, perforating form as well as its chronic form with duodenal adhesions and structures.

Seven foals, aged 18 days to three-and-a-half months, had acute necrotising and perforating duodenitis with diffuse fibrinous peritonitis. The foals came from four different farms and showed signs of illness for 0 to three days before death (five cases) or euthanasia (two cases). Clinical signs included diarrhoea, depression, distended abdomen, shock, abdominal pain, teeth grinding and sudden death. Treatment included antibiotics (three cases) and flunixin meglumine (five cases).

A further two foals showed signs of illness for 14 and 16 days respectively. These signs included diarrhoea, gastric reflux, teeth grinding, weight loss and dehydration. Both foals were destroyed after a period of treatment. In all foals, samples of intestine, lung and spleen were taken for histology, bacteriology, immunofluorescence for equine herpesvirus, and negative contrast electron microscopy for viruses, eg, adenovirus and coronavirus.

At post mortem examination, the acute cases showed necrotic grey-green friable areas in the proximal duodenum which consisted of single or multiple annular segments or long antimesenteric bands extending through the thickness of the duodenum and clearly delineated on the serosal surface. Within these areas were round or linear antimesenteric perforations. The necrotic areas were thin (1 mm) whereas the

tissue between these bands was thicker than normal.

The two chronic cases showed thickening of the duodenal wall where large areas of mucosa and submucosa were replaced by granulation tissue. In one case there were also several adhesions. In the areas of the duodenum where mucosa was present there was either moderate or severe villous atrophy with cellular infiltration or fibrosis of the lamina propria. Additional histological lesions included necrosis of lymphoid tissue of the Peyer's patches, large intestinal lymphoid nodules, mesenteric lymph nodes and spleen in all foals examined (six). One foal with serosal adhesions near the bile ducts also had diffuse acute cholangitis, subacute pancreatitis and acute erosive fibrinous inflammation of the large ducts.

Escherichia coli was isolated from intestinal contents (two cases), peritoneal fluid (one case) and various other tissues (one case). *Enterobacter* species and *Proteus* species were cultured from one foal and rotavirus from another. No equine herpesvirus, adenovirus or coronavirus was found in four cases examined.

The authors believe these to be two forms of the one syndrome and the primary lesions to be the duodenal ones because gastric ulceration is seen fairly frequently at post mortem examination of foals. The cause of the duodenitis is not known but comparisons have been made with lesions seen at *Clostridium perfringens* type B enterotoxaemia in lambs. Stress and treatment with phenylbutazone and other non-steroidal anti-inflammatory drugs may be contributory factors by creating conditions for infection to become established.

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