ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Protein Intake On the Kinetic Disposition of Intravenous Administered Gentamicin in Moroccan Sardi Breed Sheep.

Zineb Souhaili[1]*, and Mohamed Oukessou[2].

[1]Laboratory of Chemistry-Biochemistry, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca, B.P. 5696 Maarif, Casablanca, Morocco.
[2]Department of Physiology and Therapeutics, Hassan II Agronomic and Veterinary Institute, B.P. 6202, Rabat, Morocco.

ABSTRACT

The effect of three qualities of feed on the kinetic disposition of gentamicin was investigated in SARDI sheep breed (n=6). The same sheep were given sequentially three qualitatively different diets: a low-quality diet (LQ: contains 7% protein), a normal quality diet (NQ: contains 10% protein), and a high-quality diet (HQ: contains 15% protein). The three diets were based on barley, alfalfa and straw. They differ by their protein content but are energetically equivalent, During each of the three experimental phases, the animals received intravenously gentamicin, inulin and para-aminohippuric acid (PAH) respectively at doses of 4, 30 and 20 mg / kg body weight. The inulin and para-aminohippuric acid (PAH) were respectively used to estimate the glomerular filtration rate and renal plasma flow. The gentamicin, inulin and PAH concentrations were determined in blood samples taken serially from the jugular vein between 5 min and 6h after administration. The serum gentamicin concentration was determined by microbiological agar diffusion method. The results showed that the quality of feed affected the kinetic of gentamicin, the low-quality diet (LQ) led to a significant reduction in body clearance and volume distribution at steady-state of gentamicin correlated with a reduction of glomerular filtration while the high-quality diet (HQ) had no significant effect on the kinetics of gentamicin compared with normal-quality diet (NQ).

Keywords: Antibiotic, gentamicin, pharmacokinetic, sheep, protein diet

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The sheep population occupies a prominent place in the agricultural economy in Morocco. Currently, almost all of this breeding is reared exclusively in an extensive manner; it is therefore submitted to the seasonal fluctuations and circannual of the food availability. The animals are usually underfed during autumn and winter periods and well fed during spring and summer. The qualitative and quantitative variations of the dietary intake interfere with the biochemical and physiological processes of the body. Indeed, it is well known that the malnutrition reduces the metabolic and excretory capacities of the liver and the kidney functions [1, 2, 3].

The Gentamicin, an aminoglycoside antibiotic is among the most active drugs in this family. Its specter extends to numerous bacteria GRAM +, GRAM - (*E. coli* and salmonella in particular), mycoplasma and *Pseudomonas aeruginosa* [4, 5]. The gentamicin pharmacokinetics is characterized by a limited distribution at the extracellular space and elimination under active form essentially by glomerular filtration; it has a strong binding to tissue proteins [4, 6].

The objective of this study was to investigate the influence of the nitrogen intake fluctuations on the gentamicin pharmacokinetics which is widely used in veterinary medicine in Morocco among the SARDI sheep breed that occupies a prominent place in sheep farming in Morocco; moreover this breed is rustic and well adapted to the nutritional hazards of routes [7, 8]. Considering the physiological modifications inherent to the protein malnutrition, the effective renal plasma flow (ERPF), the glomerular filtration rate (GFR) and the extracellular fluid volume were also estimated.

MATERIALS AND METHODS

Animals and diets

Six adult Sardi breed ewes aged from 6 to 7 years, non-pregnant, non-lactating, clinically healthy and weighing on average 47 kg were used.

The ewes were maintained in individual metabolism cages for the duration of the experiment and received food ration of varying composition according to the experimental period

- The first period: a normal quality diet that provides 10% of proteins.
- The second period: a high quality diet that provides 15% of proteins.
- The third period: a low quality diet that provides 7% of proteins.

The three diets were based on barley grain, alfalfa and straw. They differ by their protein content but are energetically equivalent [9]. The water was given *ad libitum.*

All applicable international or institutional guidelines for the care and use of the animals were followed.

Experimental protocol

The animals underwent three experimental periods; the first period with normal diet, the second with high quality diet and the third with low quality diet.

Each experimental period was divided into two phases:

- An adaptation phase of animals to the diet of 6 weeks.
- An experimental phase using the cross-over during which animals were randomized;

One half received intravenous - administration only – of the gentamicin sulfate (Schering-Plough, Levallois-Perret, France) in injectable solution to the dose of 4 mg/kg, the other half received inulin and PAH powder (Sigma, St. Louis, MO. USA), prepared extemporaneously, and injected at the respective doses of 30 mg / kg and 20 mg / kg. The reverse protocol was performed on animals one week later.

Blood samples (5 ml) were collected by jugular venepuncture into dry vials at time 0 (before) and at 2min, 4min, 8min, 15min, 30min, 1h, 1h30min, 2h, 3h, 4h, 5h for inulin, 2 min 4 min, 8 min, 15min, 30min, 1h, 2h, 3h, 4h, 5h, 6h for gentamicin and PAH after administration of these products. The serum was separated by centrifugation (3000g) within 10 min after, divided into several fractions and stored at -25°C until analysis.

Analytical methods

The serum concentration of gentamicin was determined by microbiological method according to the agar diffusion method using *Bacillus subtilis* ATCC 6633 as microorganism test [10], each sample was analyzed in duplicate. The detection limit is 0.05µg / ml.

The inulin dosage was performed according to the colorimetric method described by Clarke et *al.* (1985) [11], and that of the PAH according to a high-performance liquid chromatography (HPLC) [12].

Pharmacokinetic analysis

The pharmacokinetic data analysis (concentrations and corresponding time) was performed using a nonlinear regression program (MULTI) [13]. The calculation of the main parameters was realized for each animal and product, according to a non-compartmental approach using the equations described in the literature [14].

The body clearance was calculated using the following equation:

$$Cl_B\,(ml/min/kg) = Dose/AUC$$

In which AUC is the area under the curve of serum concentration according to time. AUC is obtained by the arithmetic trapezoidal rule.

The volume of distribution at steady-state (Vss) was calculated according to the following equation:

$$Vss\,(l/kg) = Cl_B \times MRT$$

MRT is the mean residence time obtained using the following equation:

$$MRT\,(min) = AUMC/AUC$$

In which AUMC indicates the centred moment by first order of the curve concentration / time calculated according to the equation: $AUMC = \int_0^\infty t.C.dt$

Statistical analysis

The determination of the diet effect was realized by an analysis of the variance for all pharmacokinetic parameters calculated for each substance. The comparison of the averages was performed by the Newman-Keuls test at 5% significance level.

RESULTS AND DISCUSSION

Parameters of the water balance and renal function

The Table 1 presents the main parameters describing the kinetics of the inulin and the PAH after intravenous administration.

The body clearances of the inulin and the PAH, that estimate respectively the GFR and the ERPF, decreased during the period of the LQ diet in comparison with those of the HQ or NQ diets. In contrast, the volume of distribution at steady-state Vss of inulin, which estimates the extracellular fluid volume, was not affected by the diet.

ISSN: 0975-8585

Table 1 : Selected pharmacokinetic parameters (means ± DS) of inulin and PAH after single intravenous administration (30mg and 20mg / kg respectively) in six ewes offered normal quality (NQ), high quality (HQ) or low quality diets.

Parameters (Units)	Diets			Significance level
	NQ	HQ	LQ	
V_{SSIN} (1/kg)	0,14 ± 0,06[a]	0,12 ± 0,02[a]	0,12 ± 0,01[a]	P>0,05
Cl_{IN} (ml/min/kg)	2,13 ± 0,56[a]	2,08 ± 0,18[a]	1,23 ± 0,25[b]	P<0,05
Cl_{PAH} (ml/min/kg)	10,08 ± 1,56[a]	11,47 ± 1,82[a]	8,79 ± 0,80[a]	P=0,06

[ab]For each parameter, means with differing superscripts were statistically different between the three qualities of diets. NQ: A normal-quality diet , HQ: A high-quality diet, LQ: A low-quality diet, V_{SS} (1/kg): volume of distribution at steady-state of inulin (estimates the extracellular fluid volume) , Cl_{IN} (ml/min/kg): Corporal clearance of inulin(estimates the glomerular filtration rate), Cl_{PAH} (ml/min/kg): Corporal clearance of para-aminohippuric acid (estimates effective renal plasma flow)

Parameters of gentamicin

The mean serum concentration-time curves for gentamicin are shown in Figure 1, with the corresponding pharmacokinetic parameters given in Table 2.

Table 2: Selected pharmacokinetic parameters (mean ± DS) of the gentamicin after a single intravenous administration (4mg/kg) in six ewes offered normal quality (NQ), high quality (HQ) or low quality diets.

Parameters (Units)	Diets			Significance level
	NQ	HQ	LQ	
MRT (min)	83,70±11,82[b]	88,13±11,9[b]	106,29±11,46[a]	P≤0.05
V_{ss} (l/kg)	0,10±0,01[a]	0,09±0,02[a]	0,06±0,02[b]	P≤0.05
Cl_B (ml/min/kg)	1,23±0,19[a]	0,97±0,09[a]	0,64±0,15[b]	P≤0.05
AUC (µg.min/ml)	3329,6± 410,7[b]	4380,6±664,2[b]	7529,3±1992,7[a]	P≤0.05

[ab]For each parameter, means with differing superscripts were statistically different at p≤0.05 between the three qualities of diets. NQ: A normal-quality diet, HQ: A high-quality diet, LQ: A low-quality diet, MRT (min) : The mean residence time, V_{SS} (1/kg): The volume of distribution at steady-state, Cl_B (ml/min/kg): Corporal clearance, AUC (µg.min/ml): Area under the curve.

Figure 1 shows that at any time post-administration, the serum concentrations of the gentamicin were higher among animals submitted to low-protein diet.

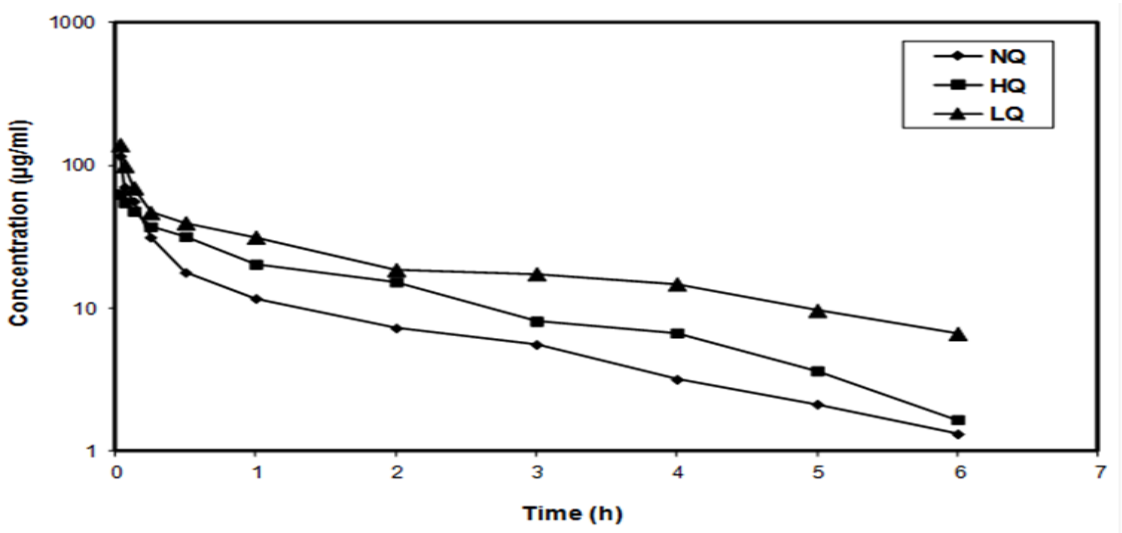

Fig 1: Mean serum concentrations of gentamicin after a single intravenous administration (4 mg / kg) in six ewes offered normal quality (NQ), high quality (HQ) or low quality (LQ) diets.

The body clearance (CLB) of the gentamicin as well as its volume of distribution at steady-state (Vss) are significantly lower ($p < 0.05$) among animals during the period of LQ compared with the NQ and HQ periods.

For a drug administered intravenously, the evolution of its serum concentration depends at the distribution process and the elimination process expressed by its clearance. The extent of the distribution of a drug is due to its physico-chemical properties, its fixation to plasma and tissue proteins, the volume of the body fluids and tissular blood flow [15].

The gentamicin is an antibiotic which the distribution is limited to the extracellular space and its volume of distribution is similar to that of the inulin [16], yet we notice that the extracellular fluid volume estimated by the Vss of inulin did not change among animals. Furthermore, the fixation of gentamicin to plasma proteins is low but it has a high affinity for the tissular proteins, it preferentially accumulates in the renal cortex, liver, lungs and ears. Therefore, the decrease in its volume of distribution would be secondary to a reduction of its tissular fixation or even to a decrease in local blood flow, in fact, the renal blood flow (estimated by the clearance of the PAH) decreased in animals under LQ.

The gentamicin is eliminated by the kidney under unchanged form, essentially by the glomerular filtration [17, 4]. its clearance is similar to FGR or even to inulin clearance, it is likely that the decrease of elimination of the gentamicin in animals under LQ is secondary to the decrease of the FGR.

The decrease of the body clearance and the Vss of gentamicin has increased significantly the MRT and the AUC of this substance, yet the gentamicin is an antibiotic which has a dose-dependent activity, therefore, in case of repeated administrations its serum concentration will be higher and sustained over time in animals under the LQ diet which will have for consequence a potentiating of the therapeutic activity and as well toxic of this drugs especially that this one has a low therapeutic index, it is deposited on the ears and kidneys resulting of toxic effect to animals [18, 6, 19].

In terms of residues, the protein undernutrition would favor an increase of the residues gentamicin in products of animal origin destined for human consumption. However, apart from a possible effect on the intestinal flora, the danger of the residues gentamicin among the consumer would have a little importance in so far as this antibiotic is weakly absorbed by the digestive tract [20, 21].

CONCLUSION

Based on these results that show a change in the pharmacokinetics of gentamicin among animals under low-protein diet, and knowing that the majority of the Moroccan sheep population is raised in extensive mode and thus subjected to the malnutrition related to the fluctuations in rainfall relating to climate change.

The observed effects will depend on the severity of undernutrition, especially that during drought periods, the animals are subjected to both protein and energy malnutrition.

Hence a simple extrapolation of dosing regimens established without considering the nutritional state of the animal could lead to therapeutic failures and / or toxicity, especially with low therapeutic index drugs.

Ethical approval: All applicable international or institutional guidelines for the care and use of the animals were followed.

ACKNOWLEDGEMENTS

The authors acknowledge the Mr. Mohamed Ablouh, and Mr. Said Suilahi (Hassan II Agronomic and Veterinary Institute) for technical assistance and thank Miss Rachad Chafik El Idrissi for revising the manuscript.

REFERENCES

[1] Kazue Fugihara C, Marcondes M, Zatz R. Ren Physiol Biochem. 1990; 13(4): 206-212.

[2] Martin WF, Armstrong LE, Rodrguez NR. Nut Metabol. 2005; 2: 25-35.
[3] Ambuhl BM. Inter J Vitam Nut Res. 2011; 81 (2-3): 162-172.
[4] Prescott JF. In: Prescott JF, Baggot JD, Walker RD, (Eds), 3rd ed. Ames, Iowa: Iowa State Univ Pr, (2000); 191–228.
[5] Abu-basha E, Idkaidek NM, Al-shunnaq AF. Vet Res Comm. 2007; 31(6): 765-773.
[6] Giguère S, Prescott J F, Baggot D, Walker R D, Dowling PM. In: Veterinary Medicine 4th ed. Blackwell Publishing, 2007; 626 p.
[7] Boujnane I. Actes de l'I.A.V Hassan II, Rabat. 2003; 2: 14-48.
[8] El fadili M. Bull Transf Technol Agric. 2000; 149**:** 1-4.
[9] Guerouali A. (PhD thesis). Animal Sciences department, Colorado state University Fort collins USA, 1990; 242p.
[10] Edberg SC (1986). In: Antibiotics in Laboratory Medicine (Lorian V, ed) Williams and Wilkins Los angelos, 1986; 381-476.
[11] Clarke CR, SHORT CR, Hsu RC, Baggot JD (1985). Am J Vet Res.1985; 2461-6.
[12] Oukessou M, Toutain PL (1992). J Vet Pharmacol and Therap.1992; 15: 421-32.
[13] Yamaoka K, TANIGAWARA Y, Nakagawa T, Uno T (1981). J Pharmaco-biodyn. 1981; 4: 879-885.
[14] Gibaldi M, Perrier D. 2nd Ed. Marcel Dekker New York, 1982; 482p.
[15] Baggot JD J Am Vet Med Assoc. 1980; 176 (10):1086-90.
[16] Rivière JE, Bowman KF Rogers RA. J Pharmacol ExperTherap.1985; 234: 90-3.
[17] Benitz AM (1984). J Am Vet Med Assoc.1984; 185 (10): 1118-23.
[18] Zeeni N, Selmaoui B, Beauchamp D, Labresque G, Thibault L. Physiol. Behav. 2007; (90): 760-770.
[19] Fartashvand M, Mousavi G, Hajisadeghi Y (2014). Bull Vet Ins. Pulawy. 2014; 58: 657-661.
[20] Ristuccia AM. In: Atimicrobial therapy. Ristuccia AM and Cunha BA, *(*Eds) Raven press. New York, 1984; pp 305-325.
[21] Brisabois A, Kempf I, Meunier D, MillemannY, Sanders P, Toutain PL. Eds Groupe de travail (Président Guillemot D). Maisons-Alfort: AFSSA, 2006. 214p.