

## Effect of Intramuscular Injection of Tobramycin on Some Biochemical Parameters in Blood of Sheep

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**Abstract:** The object of the present study was to assess the possible alterations in blood of sheep that may occur following intramuscular injection of tobramycin. Tobramycin was injected to 10 sheep at a dosage regimen of 5 mg/kg of body weight for 5 successive days. Two types of blood samples (with and without EDTA as an anticoagulant) were collected from the jugular vein before and after the antibiotic course. Tobramycin significantly ( $p < 0.05$ ) increased levels of blood urea nitrogen, creatinine, aspartate aminotransferase, alkaline phosphatase, sodium and calcium. On the other hand, it significantly ( $p < 0.05$ ) decreased serum total bilirubin level. Other parameters including, erythrocytic count, packed cell volume, hemoglobin, total and differential leukocytic counts, alanine aminotransferase, G-glutamyltransferase, potassium and phosphorus did not show any significant difference when compared with those of prior-administration samples. These data suggest that tobramycin should be used with care in treatment of sheep with renal impairment; its dosage regimen should be adjusted to avoid its nephrotoxic effect.

**Key words:** Biochemical parameters, blood, hepatotoxicity, nephrotoxicity, sheep, tobramycin

### INTRODUCTION

Since the discovery of antibiotics, various investigations have been carried out to reveal their pharmacodynamic profile and side effects. Although antibiotics are generally considered safe and well tolerated, they have been associated with a wide range of adverse effects (Slama *et al.*, 2005). Side effects are many, various, and can be very serious depending on the antibiotics used and the microbial organisms targeted. Pharmacodynamic effects of antibiotics on blood constituents, serum enzymes and electrolytes were extensively studied in laboratory and in domesticated animals (Jernigan *et al.*, 1988; Lin *et al.*, 1996; Lashev and Lasarova, 2001; AL-Motabagani, 2007; Dinev *et al.*, 2007).

Tobramycin is one of the most active antibiotics of the chemically related aminoglycosides. It is obtained from *Streptomyces tenebrarius* and acts primarily by disrupting protein synthesis of bacterial cell, leading to altered cell membrane permeability. Tobramycin can be used clinically to treat serious Gram-negative and some aerobic Gram-positive infections in most species including camel (Al-Hadi *et al.*, 2008), but the most important property of this antibiotic is its activity against most strains of *Pseudomonas aeruginosa*. As other

aminoglycosides it is mainly eliminated through glomerular filtration, although aminoglycosides are widely used in clinical practice, serious toxicity is a major limitation to the usefulness of their use, as nephrotoxicity, ototoxicity, neuromuscular blockade and hypersensitivity (Hoffmann *et al.*, 2002; Olsen *et al.*, 2004) which limits their systemic use for serious infections, although it is usually reversible once the drug is discontinued. Whether tobramycin is less nephrotoxic than other members of the aminoglycosides when used clinically is still controversial (Plumb, 2008). However drug residence and prolonged meat withholding times make the use of these antibiotics is prohibited in food animals (Mohler *et al.*, 2009).

Comparative investigations on changes in biochemical and haematological parameters after tobramycin, amoxicillin and other antibiotics in the same groups are scarce in ruminants (Dinev *et al.*, 2007; Lashev and Lasarova, 2001; Sumano *et al.*, 2005). Since antibiotics are used for treatment of infections that may also cause changes in some haematological and biochemical parameters, the physician must take into consideration changes caused by these drugs to avoid an incorrect diagnosis.

Therefore, this study was designed to investigate the possible haematological and biochemical changes in

Table 1: Haematological changes in blood picture of sheep injected with tobramycin (5 mg/kg of body weight) intramuscularly, twice daily for 5 successive days (Data are expressed as mean±S.E; n = 10)

	RBCsx10 <sup>6</sup> (Cumm)	Hb (gm/dL)	PCV (%)	WBCsx10 <sup>3</sup> (Cumm)	Differential leukocytic count				
					Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Control	12.9±0.41	12.4±0.32	37.3±1.02	7.45±0.19	40.8±2.59	3.9±0.48	0.3±0.14	49.8±2.83	3.2±0.37
Tobramycin	12.7±0.45	12.2±0.35	36.9±1.12	7.55±0.26	42.2±1.6	3.6±0.63	0.3±0.14	50.7±1.71	3.2±0.37

healthy Libyan sheep after parenteral administration of tobramycin in therapeutic dose for five successive days.

### MATERIALS AND METHODS

**The drug:** Tobramycin sulphate (Nebcin)<sup>®</sup>: Obtained in the form of vials of 2 mL, each mL contains 80 mg Tobramycin sulphate, manufactured by Sothema, Morocco under license, Eli Lilly, Geneva.

**Experimental animals:** A total of 10 Libyan, apparently healthy, fat-tailed sheep aged from 10-12 months, belong to the Faculty of Veterinary Medicine, Tripoli University were used in this study. Animals were kept in a semi-closed system under natural day light and temperature, fed on standard ration of hay and concentrate *ad libitum* and had free access to water. Animals were free from ectoparasites and blood parasites.

**Experimental design:** Animals were weighed individually at the beginning of the experiment; blood samples were taken from each animal and considered as control.

On the next day animals were injected with the therapeutic dose (5 mg/kg B. Wt.) of tobramycin sulphate, twice daily, for 5 successive days.

At the end of the experiment, 2 h after the last injection, blood samples were taken from all animals for biochemical and haematological investigation.

**Samples:** Two blood samples were collected from the jugular vein of each sheep, one is introduced into a plane glass tube to obtain serum while the other to a glass tube containing EDTA for haematological examination.

**Haematological examination:** Total Red Cell Count (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV) and total White Blood Cell count (WBC) were determined manually according to Schalm *et al.* (1975). Blood smears were prepared, fixed and stained with May-Grunwald-Giemsa stain, 200 leukocyte were differentiated in blood smear of each animals.

### Biochemical examination:

**Kidney function tests:** Blood Urea Nitrogen (BUN) was estimated using urea kits (Bio Meriux) according to Patton and Crouch (1977). Serum Creatinine (SCr) was determined using creatinine kit (Bio Meriux) according to

Husdan and Rapoport (1968). Serum calcium, phosphorous, sodium and potassium were measured using flame photometer according to Sarkar and Chauhan (1967), Zilversmit and Daris (1950) and Oser (1979), respectively.

**Liver function tests:** The level of Alanine amino Transferase (ALT) and Aspartate amino Transferase (AST) were determined using transaminase kits (Bio Meriux) according to Reitman and Frankel (1957). Alkaline Phosphatase (ALP) and Gamma Glutamyl Transferase (GGT) levels was estimated according to Roy (1970) and Szas (1974), respectively. Total Bilirubin (TB) was measured with a colorometric method using Boehringer Mannheim GmbH kits, according to Jendrassik and Grof (1938).

**Statistical analysis:** Results were statistically analyzed using student's t-test after Snedecor and Cochran (1973).

Table 2: Response of blood urea nitrogen and serum creatinine levels of sheep injected with tobramycin (5 mg/Kg of body weight) intramuscularly twice daily for 5 successive days (Data are expressed as Mean±S.E; n = 10)

	Control	Tobramycin
Blood urea nitrogen (mg/100 mL)	29.7±2.46	36.8±2.75*
Serum creatinine (mg/100 mL)	0.76±0.03	0.81±0.03*

\*: Significance p<0.05

Table 3: Response of sheep serum enzyme levels to tobramycin (5 mg/Kg of body weight), injected intramuscularly twice daily for 5 successive days (Mean±S.E; n = 10)

	Control	Tobramycin
ALT (U/L)	16.7±1.99	14.3±2.24
AST (U/L)	64.8±3.6	78.8±3.2**
GGT (U/L)	33.5±2.16	35.0±2.2
ALP (U/L)	284.2±19.6	365.0±24.5**
Cholesterol (mg/dL)	66.5±5.27	64.6±4.86
Total bilirubin (mg/dL)	0.45±0.025	0.35±0.02**

\*\* : Significance p<0.01

Table 4: Response of sheep serum electrolyte levels to tobramycin (5 mg/Kg of body weight), injected intramuscularly twice daily for 5 successive days (Mean±S.E; n = 10)

	Control	Tobramycin
Sodium (mg/100 mL)	139.0±2.79	146.1±0.75**
Potassium (mg/100 mL)	5.27±0.27	5.56±0.37
Calcium (mg/100 mL)	8.74±0.29	9.57±0.29*
Phosphorus (mg/100 mL)	9.07±0.65	8.28±0.62

\*: Significance p<0.05; \*\*: Significance p<0.01

## RESULTS

Blood picture for experimental animals at zero time (control) and after treatment with tobramycin are shown in Table 1, sheep injected with tobramycin, showed no statistical difference in any of the blood parameters when compared with the control.

Results for serum biochemistry applied to evaluate liver and kidney function after 5 days of treatment with the drug are shown in Table 2 and Table 3. When compared with the control, a significant ( $p < 0.01$ ) increase in AST and ALP has been detected together with a decrease in Total Bilirubin (TB) in animals after tobramycin.

A significant ( $p < 0.05$ ) increase in BUN (Table 2) and calcium (Table 4), with a significant ( $p < 0.01$ ) increase in SCr (Table 2) and sodium (Table 4) has been detected.

## DISCUSSION

Tobramycin is a member of the aminoglycoside antibiotics, these potent antibiotics are commonly used to treat serious infections caused by many Gram-negative bacilli and some Gram-positive organisms in man and small animals (Al-Motabagani, 2007; Chaudhary *et al.*, 2009). Drug residence and prolonged meat withholding times make the use of aminoglycosides in well-developed countries prohibited in food animals (Mohler *et al.*, 2009).

In this study, blood parameters in the tobramycin-treated animals showed no statistical differences when compared with the control, this result was in agreement with Lorin *et al.* (1980). In contrast, Dinev *et al.* (2007) has detected a significant decrease in RBC, Hg, PCV and total WBC count in goats treated parentally with the same dose of tobramycin once daily for 5 days. Goat RBCs have a long life span, so it is unexpected for tobramycin to cause a decrease in erythrocytic parameters after such short course of treatment unless it is caused by a haemolytic mechanism which was not a problem in this work.

ALT, a hepatic leakage enzyme did not show significant change after tobramycin treatment, because its activity is not high in ruminant hepatocytes (Bain, 2003). In addition to that, the significant increase in ALP monitored in the same animals was not associated with an increase in bilirubin. It is obvious that ALP is a cholestatic marker and its synthesis and release increases in association with impaired bile flow (Bain, 2003) and the total bilirubin was significantly lower than the control after tobramycin; accordingly, this increase in ALP may be considered non specific and attributed to its wide and fluctuating reference intervals in ruminants (Bain, 2003; Dinev *et al.*, 2007). In the same time, GGT the specific

cholestatic enzyme in large animals showed no significant changes after the drug treatment. The significant decrease in bilirubin level after tobramycin may be explained by the tobramycin-induced increase in bile secretion through increasing glycosyl derivatives in hepatic microsomes that increases bilirubin conjugation and clearance (Cornelius, 1989). In general, aminoglycosides are excreted in small part through the liver and its concentration in the bile is about 30% of the blood level (Katzung, 1995). Severe to mild transient hepatotoxicity induced by tobramycin and other aminoglycosides treatment has been reported in man (Nisly *et al.*, 2007), while Chaudhary *et al.* (2009) found no significant change in liver and kidney function tests in patients with lower respiratory tract infection treated with a combination of tobramycin and ceftazidime. An increase in ALP and HDL has been detected in goats after tobramycin treatment (Dinev *et al.*, 2007).

Tobramycin caused a significant increase in SCr and BUN in comparison with the pre-treatment values, this increase in non-protein nitrogenous substances indicates a decrease in GFR, this finding is in agreement with the finding of many other workers that attribute these changes to nephrotoxicity induced by tobramycin (Aronoff *et al.*, 1990; Katzung, 1995; Dinev *et al.*, 2007; Plumb, 2008). In this experiment, the increase in SCr is not considered significant because it remained within normal reference range for this animal species, which means that the kidneys in these animals are still capable of creatinine excretion; unlike BUN which was above the normal value of that animal species and significantly higher than the control. In ruminants, increase in SCr is more specific than BUN to detect a decrease in GFR, since in these unique animals, ammonia derived from urea is utilized to form amino acids (Kaneko *et al.*, 2008), so this increase in BUN may be in part due to increase in protein catabolism rather than nephrotoxicosis (Shils, 1963). In addition to that, SCr is freely filtered from the glomeruli and it is not reabsorbed by renal tubules, unlike urea that passively diffuses from the tubules back to the body and the amount of urea absorbed is inversely related to the rate of urine flow through the tubules (Kaneko *et al.*, 2008), this may further explain the more significant increase in BUN than SCr in our study. Nephrotoxicity resulting from tobramycin is characterized by tubular damage, with most of the changes confined to the proximal convoluted tubules (Kaloyanides and Pastoriza-Munoz, 1980; Al-Motabagani, 2007).

The kidneys are involved in the homeostasis of calcium, phosphorous, sodium, calcium and potassium (Mayer and Harvey, 2004). In this study, the higher sodium level in the tobramycin group run parallel with the increase in BUN, as both are freely filtered from the glomeruli. In renal tubular diseases, hyponatraemia is

expected due to increase in fractional excretion of sodium (Mayer and Harvey, 2004). Anyhow, the level of sodium was within normal reference range and this increase may also be attributed to normal physiological fluctuation. Hypocalcaemia and hyperphosphataemia (Mayer and Harvey, 2004) were detected in animals before and after treatment (Table 2), so it cannot be attributed to renal malfunction (Mayer and Harvey, 2004). The level of calcium after tobramycin treatment was significantly higher than that of the control; the changes in these two electrolytes may not match with abnormal renal function.

It could be concluded that tobramycin given in therapeutic doses twice daily to healthy Libyan sheep caused only minor inconclusive changes in the haematological and biochemical profiles in treated animals; and thus can be used safely in treating different susceptible bacterial infections in sheep provided that the kidney and liver are functioning normally.

#### REFERENCES

- Al-hadi, A., I.A. Wasfi, F.A. Gadir, M.H. Amiri, A.K. Bashir and J.D. Baggot, 2008. Pharmacokinetics of tobramycin in camels. *J. Vet. Pharmacol. Ther.*, 17: 48-51.
- Al-Motabagani, M., 2007. Histopathological study on the effect of tobramycin dosage regimens on renal proximal tubular cells in the rats. *Pak. J. Med. Sci.*, 23: 71-77.
- Aronoff, G., G. Brier, R. Sloan and M. Brier, 1990. Interactions of ceftazidime and tobramycin in patients with normal and impaired renal function. *Antimicrob. Agents Chemother.*, 34: 1139-1142.
- Bain, V.G., 2003. Hepatorenal syndrome, hepatopulmonary syndrome and now, hepatospinal syndrome? *Liver Transpl.*, 9: 995-6.
- Chaudhary, M., S. Shrivastava and R. Sehgal, 2009. Evaluation of the efficacy and safety of fixed dose combination of ceftazidime-tobramycin in comparison with ceftazidime in lower respiratory tract infections. *Curr. Clin. Pharmacol.*, 4: 62-66.
- Cornelius, C.E., 1989. Liver Function. In: *Clinical Biochemistry of Domestic Animals*. 4th Edn., Academic Press, New York.
- Dinev, T., D. Zapryanova and L. Lashev, 2007. Changes in some blood biochemical and haematological parameters in goats after aminoglycoside and aminocyclitol treatment at therapeutic doses. *Turk. J. Vet. Anim. Sci.*, 31: 179-188.
- Hoffmann, I.M., B.K. Rubin, S.S. Iskandar, M.S. Schechter, S.K. Nagaraj and M.M. Bitzan, 2002. Acute renal failure in cystic fibrosis: Association with inhaled tobramycin therapy. *Pediatr. Pulmonol.*, 34: 375-377.
- Husdan, H. and A. Rapoport, 1968. Estimation of Creatinine by the Jaffe Reaction. *Clin. Chem.*, 14: 222-238.
- Jernigan, A.D., R.C. Hatch and R.C. Wilson, 1988. Pharmacokinetics of tobramycin in cats. *Am. J. Vet. Res.*, 49: 608-612.
- Jendrassik, L. and P. Grof, 1938. Vereinfacht photometrische Methoden zur Bestimmung des Butyliribins. *Biochemistry*, 3: 297.
- Kaloyanides, G.J. and E. Pastoriza-Munoz, 1980. Aminoglycoside nephrotoxicity. *Kidney Int.*, 18: 571-582.
- Kaneko, J.J., J.M. Harvey and M.L. Bruss, 2008. *Clinical Biochemistry of Domestic Animals*. 6th Edn., Academic Press, San Diego, USA.
- Katzung, B.G., 1995. *Basic and Clinical Pharmacology*. Connecticut: Appleton and Lange, country, pp: 680-699.
- Lashev, L. and S. Lasarova, 2001. Pharmacokinetics and side-effects of gentamicin in healthy and pseudomonas aeruginosa infected sheep. *J. Vet. Pharmacol. Ther.*, 24: 237-240.
- Lin, L., L. Grenier, M. Leburn, M. Bergeron, L. Thibault, G. Labrecque and D. Beauchamp, 1996. Day-Night treatment difference of tobramycin serum and intrarenal drug distribution and nephrotoxicity in rats: Effects of fasting. *Chronobiol. Int.*, 13: 113-121.
- Lorin, D., J. Jahn and M. Kopschitz, 1980. Blood picture of infertile mares during treatment with penicillin, ampicillin, chloramphenicol or neomycin. *Wiener Tierärztliche Monatsschrift*, 67: 206-212.
- Mayer, D.J. and J.W. Harvey, 2004. *Veterinary Laboratory Medicine, Interpretation and Diagnosis*. Saunders, Philadelphia.
- Mohler, V.L., M.M. Izzo and J.K. House, 2009. Salmonella in calves. *Vet. Clin. North. Am. Food Anim. Pract.*, 25: 37-54.
- Nisly, S., S. Ray, R. Moye, E. Soler and M. Larouche, 2007. Tobramycin-induced hepatotoxicity. *Ann. Pharmacother.*, 41, 2061-2065.
- Olsen, K.M., M.I. Rudis, J.A. Rebeck, J. Hara, D. Belmont, R. Mehdian, C. Nelson and M.E. Rupp, 2004. Effect of once-daily dosing vs. multiple daily dosing of tobramycin on enzyme markers of nephrotoxicity. *Crit. Care Med.*, 32: 1678-1682.
- Oser, B.L., 1979. *Howk's Physiological Chemistry*. 14th Edn., Tata McGraw Hill, Publishing Company Limited and Primate by Mohan-Makijant at Rekha Printers Pvt., Ltd., New Delhi, 110020.
- Patton, C.J. and S.R. Crouch, 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonium. *Anal. Chem.*, 49: 464-469.
- Plumb, D.C., 2008. *Veterinary Drug Handbook*. 6th Edn., Distributed by Blackwell Pub., Stockholm, Ames, Iowa.

- Reitman, S. and S. Frankel, 1957. Colorimetric method for the determination of serum transaminase activity. *Am. J. Clin. Pathol.*, 28: 56-58.
- Roy, A.V., 1970. A rapid method for alkaline phosphatase estimation. *Clin. Chem.*, 16: 431.
- Sarkar, B.C. and U.P. Chauhan, 1967. A new method for determining micro quantities of calcium in biological materials. *Anal. Biochem.*, 20: 155-166.
- Schalm, O.W., N.C. Jain and E.G. Carroll, 1975. Normal values in blood morphology with comments on species characteristics in response to disease. In *Veterinary Haematology*, pp: 82.
- Shils, M.E., 1963. Renal disease and the metabolic effects of tetracycline. *Ann. Intern. Med.*, 58: 389-408.
- Slama, T.G., A. Amin, S.A. Brunton, T.M.J. File, G. Milkovich, K.A., Rodvold, D.F. Sahn, J. Varon and D.J. Weiland, 2005. A clinician's guide to the appropriate and accurate use of antibiotics: The council for appropriate and rational antibiotic therapy (CARAT) criteria. *Am. J. Med.*, 118(7): 1-6.
- Snedecor, G.W. and G.W. Cochran, 1973. *Statistical Methods*. 6th Edn., Iowa state University Press, USA.
- Sumano, H., L. Gutierrez, C. Velazquez and S. Hayashida, 2005. Pharmacokinetics and renal toxicity of three once-a-day doses of amikacin in cows. *Acta. Vet. Hung.*, 53: 231-240.
- Szasz, G., 1974. *Method of Enzymatic Analysis*. Bergmeyer, H.U., (Ed.), 3rd Edn., Verlag Chemie Weinheim, pp: 757-762.
- Zilversmit, D.B. and K.J. Daris, 1950. Micro-determination of plasma phospholipids by TCA precipitation. *Lab. Clin. Med.*, 35: 155-161.