# EVALUATION OF SAFETY OF TOBRAMYCIN AND PIPERACILLIN COMBINATION ON RABBIT KIDNEY

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*Tobramycin, an aminoglycoside, and piperacillin, an antipseudomonal penicillin, are widely used to treat Gram –ve infections. Their concurrent use is recommended because of their synergistic action and prevention of resistant strains against monotherapy. This experimental work was aimed to study the effects and interaction of these important drugs on rabbit kidney. Male rabbits were administered tobramycin, piperacillin, and tobramycin plus piperacillin for 21 days (n = 6 in each group). Blood and urine samples were collected on day 0, 11, and 21. Blood was analyzed for BUN, serum creatinine, serum potassium, and serum sodium while urine was analyzed for urine volume, creatinine, albumin and specific gravity. Renal creatinine clearance was calculated. Results showed some change in renal function with use of tobramycin but piperacillin neither changed the renal function nor did augment the toxic effect of tobramycin so it was concluded that combination of both drugs in safe.*

Bacterial infections remain a major cause of illness and death throughout the world. Various antibiotics have been used to treat such infections effectively. Among these aminoglycosides and penicillins are commonly used for serious bacterial infections. Tobramycin being an aminoglycoside is frequently used for the therapy of proven or suspected serious Gram negative microbial infections, specially those due to Pseudomonas aeruginosa, Enterobacter, Klebsiella, Serratia. Among these are urinary tract infections, bacteraemia, infected burns, osteomyelitis, pneumonia, peritonitis[1].

The aminoglycosides are bactericidal in action by virtue of irreversible inhibition of protein synthesis in susceptible microorganisms[2]. It has been reported that ureidopenicillins especially piperacillin has better activity against Enterococci, Klebsiella and Pseudomonas aeruginosa. Marked reduction in hospital surgical infection was seen when piperacillin was used in gynaecological prophylaxis [3] and it was comparable to clindamycin plus gentamicin in treatment of surgical patients with intraabdominal infection[4]. Piperacillin is also proposed as first line treatment for neutropenic patients with fever[5].

Pseudomonas aeruginosa has emerged as an important bacterial pathogen associated with substantial mortality. Combination of antipseudomonal penicillin with aminoglycoside is quite an effective treatment. It was described by Chatzinidolaou that the combination of an aminoglycoside with beta-lactum resulted in an enhanced cure rate in patients with pseudomonal infection compared to aminoglycoside monotherapy[6]. This is due to the face that beta-lactam antibiotics exhibit synergy with aminoglycosides thereby decreasing the inhibitory concentration of these agents[7]. Transport of aminoglycosides may be enhanced by cell active agents such as penicillin or vancomycin which may be basis of synergy[8]. This is an example of useful interaction of drugs. The outcome may be harmful if the interaction causes an increase in toxicity of drugs.

Studies have shown that all aminoglycosides have the potential to produce reversible and irreversible vestibular, cochlear and renal toxicity[9]. Of the beta-lactam antibiotics, several cephalosporins and a single penicillin, guanyl ureidopenicillin have been reported to have direct or nonimmunological nephrotoxic potential[10]. The nephrotoxic effect of gentamicin and tobramycin can be increased by concurrent use of cephalothin. So doses of drugs which are well tolerated separately can be nephrotoxic when given together[11].

Keeping in view these observations we decided to study the effect of combined use of piperacillin and tobramycin on renal function and compared with the drugs used alone.

## MATERIALS AND METHODS

The study was conducted on 18 male rabbits weighing 1-1.5 kg. They were divided into 3 groups of six rabbits each. Group T received tobramycin sulphate 40 mg/kg/day in two divided doses i.m. for 21 days. Group P received peperacillin 250 mg/kg/day in two divided doses i.v. for 21 days. Group TP received both drugs in above mentioned doses for the same period. Blood and urine sam-

ples were collected on day 0, 11 and 21. Blood was taken from ear vein after making it prominent with xylene. It was centrifuged and serum was stored at 4°C for renal function test i.e. urea, creatinine and electrolytes i.e. $Na^+$ and $K^+$. Urine was collected by keeping each rabbit separately in metabolic cage for 24 hrs. Urinary output for 24 hrs was measured and urine creatinine, albumin and specific gravity were estimated. Renal creatinine clearance was calculated from the above data. Commercial preparations of drugs used were inj. Nebcin containing 80 mg tobramycin sulphate in 2 ml. by Lilli and inj. Tazocin containing 4g peperacillin and 500 mg tazobactam by Wyeth.

Results were described as mean ± SEM. Values at day 0 were taken as control for each group and results on day 11 and 21 were compared with control by using Students' *t-test*.

## RESULTS

### Tobramycin

*Blood*

Blood urea nitrogen increased steadily from initial value of 20.75±3.00 to 25.00±2.38 on day 11 to 27.70±1.32 on day 21. Increase in BUN was significant from day 0 to day 11 (P<0.03) and from day 0 to 21 (P<0.01). Serum creatinine level was increased from initial level of 1.142±0.021 to 1.171±0.016 on day 11 to 1.218 on day 21. Change was significant from day 0 to day 21 (P<0.03). Significant decrease in serum sodium was seen from day 0 to day 21 136.33±0.654 to 134.33± 1.333 (P<0.01). A decrease in serum potassium level was observed in tobramycin treated group from initial value of 4.38±0.54 to 4.13±0.06 on day 11 and 3.62±0.10 on day 21 (Table 1).

***Urine***

In tobramycin group, urine volume was increased from day 0 to day 11 from 71.33±4.31 to 82.33±9.32 but the increase was not statistically significant however urine volume from day 11 to 21 was decreased from 82.33±9.32 to 52.50±3.63 which was statistically significant (P<0.02). When compared from day 0 to 21 again there was decrease in urinary output with statistically significant (P<0.02). Traces of urinary albumin were observed in 33.3% of tobramycin treated animals on day 11. In the same group albumin was positive in 33.3% while 33.3% showed traces on day 21. (fig.1&2) (Creatinine clearance in the same group reduced from day 0 to 11 from 2.43±0.44 to 1.13±0.08 (P<0.02). It continued to decrease from day 11 to 21 with values of 1.13±0.08 to 0.46±0.09 (P<0.01). Decrease was also statistically significant from day 0 to 21 (P<0.01). Specific gravity increased from day 0 to 11 from 1.001 to 1.007 (P<0.01). Increase in specific gravity was also seen from initial value of 1.001 on day 0 to 1.006±0.002 on day 21 (P<0.02) (Table 2).

**Table 1:** *Comparison of Tobramycin (T) group for blood related tests at day-0, day-11 and day-21.*

| | Day-0 | Day-11 | Day-11 | Day-21 | Day-0 | Day-21 |
|---|---|---|---|---|---|---|
| **BUN (mg/dl)** | 20.75 ±3.00 | 25.00 ±2.38 | 25.00 ±2.38 | 27.70 ±1.32 | 20.75 ±3.00 | 27.70 ±1.32 |
| | P-value < 0.03 | | P-value = 0.07 | | P-value < 0.01 | |
| **Serum Creatinine (mg/dl)** | 1.142 ±0.021 | 1.171 ±0.016 | 1.171 ±0.016 | 1.218 ±0.043 | 1.142 ±0.021 | 1.218 ±0.043 |
| | P-value = 0.11 | | P-value = 0.09 | | P-value < 0.03 | |
| **Serum Sodium (mEq/l)** | 136.33 ±0.654 | 139.83 ±1.167 | 139.83 ±1.167 | 134.33 ±1.333 | 136.33 ±0.654 | 134.33 ±1.333 |
| | P-value = 0.18 | | P-value = 0.09 | | P-value < 0.01 | |
| **Serum Potassium (mEq/l)** | 4.38 ±0.547 | 4.13 ±0.067 | 4.13 ±0.067 | 3.62 ±0.102 | 4.38 ±0.547 | 3.62 ±0.102 |
| | P-value < 0.04 | | P-value < 0.01 | | P-value < 0.01 | |

Results represent mean and ± standard error of mean

**Table 2:** *Comparison of tobramycin (T) group for urine related tests at day-0, day-11 and day-21.*

| | Day-0 | Day-11 | Day-11 | Day-21 | Day-0 | Day-21 |
|---|---|---|---|---|---|---|
| **Urinary Output in 24 Hours (ml)** | 71.33 ±4.31 | 82.33 ±9.32 | 82.33 ±9.32 | 52.50 ±3.63 | 71.33 ±4.31 | 52.50 ±3.63 |
| | P-value = 0.12 | | P-value < 0.02 | | P-value < 0.02 | |
| **Creatinine Clearance (ml/min)** | 2.43 ±0.44 | 1.13 ±0.08 | 1.13 ±0.08 | 0.46 ±0.09 | 2.43 ±0.44 | 0.46 ±0.09 |
| | P-value < 0.02 | | P-value < 0.01 | | P-value < 0.01 | |
| **Specific Gravity** | 1.001 ±0.001 | 1.007 ±0.001 | 1.007 ±0.001 | 1.006 ±0.002 | 1.001 ±0.001 | 1.006 ±0.002 |
| | P-value < 0.01 | | P-value = 0.35 | | P-value < 0.02 | |

Results represent mean and ± standard error of mean

**Table 3:** *Comparison of piperacillin (P) groups for blood related test at day-0, day-11 and day-21.*

| | Day-0 | Day-11 | Day-11 | Day-21 | Day-0 | Day-21 |
|---|---|---|---|---|---|---|
| **BUN (mg/dl)** | 21.82 ±0.94 | 21.56 ±1.63 | 21.56 ±1.63 | 22.76 ±1.03 | 21.82 ±0.94 | 22.76 ±1.03 |
| | P-value = 0.42 | | P-value = 0.11 | | P-value = 0.18 | |
| **Serum Creatinine (mg/dl)** | 1.16 ±0.09 | 1.55 ±0.07 | 1.55 ±0.07 | 1.68 ±0.07 | 1.16 ±0.09 | 1.68 ±0.07 |
| | P-value < 0.02 | | P-value < 0.02 | | P-value < 0.01 | |
| **Serum Sodium (mEq/l)** | 137.67 ±1.58 | 137.00 ±1.53 | 137.00 ±1.53 | 136.00 ±1.37 | 137.67 ±1.58 | 136.00 ±1.37 |
| | P-value = 18 | | P-value = 0.04 | | P-value = 0.07 | |
| **Serum Potassium (mEq/l)** | 4.13 ±0.11 | 3.77 ±0.08 | 3.77 ±0.08 | 3.58 ±0.10 | 4.13 ±0.11 | 3.58 ±0.10 |
| | P-value < 0.01 | | P-value < 0.01 | | P-value < 0.01 | |

Results represent mean and ± standard error of mean

**Piperacillin**

***Blood***

Blood urea nitrogen and serum sodium levels did not change significantly during the study period, however, serum creatinine was increased from initial value of 1.16±0.09 to 1.55±0.07 on day 11 and 1.68±0.07 on day 21, which was significant (P<0.01). Serum potassium level was decreased from initial value of 4.13±0.11 to 3.77±0.08 on day 11 and it continued to decrease to a value of 3.58±0.10 on day 21, which was statistically significant [P<0.01] (Table 3).

***Urine***

In piperacillin, treated group urinary output, creatinine clearance and specific gravity did not change significantly during the study period (Table.4). In 50% of treated animals traces of albumin were observed while 50% showed positive test for albumin on day 11. Results for albumin were same on day 21 (fig. 1 and 2).

**Tobramycin/Piperacillin**

***Blood***

Blood urea nitrogen was increased from initial

value of 20.99±1.30 to 25.42±1.31 on day 11 (P<0.01). On day 21 it reduced slightly but it was not significant. Serum creatinine was increased from initial value of 1.21±0.08 on day 0 to 1.46±0.05 on day 11 (P<0.01) and then on day 21 1.58±0.04 (P<0.01). Serum sodium was reduced from day 0 to day 11 values 133±2.11 to 129±0.86 (P<0.04). However serum potassium showed a steady decrease throughout the study period. Initial value 4.18±0.13, on day 11, 3.45±0.10 (P<0.01) and on day 21, 3.82±0.05 [P<0.01] (Table 5).

***Urine***

In tobramycin/piperacillin increase in urine output was seen from day 0 to day 21, values being 19.17±2.48 to 26.33±2.81 was statistically significant (P<0.02). Creatinine clearance was reduced form initial value of 1.95±0.47 to 0.96±0.22 on day 11 but an increase in creatinine clearance was seen from day 11 to day 21 that was statistically significant (P<0.03).(Table 6) On day 11 traces of albumin were observed in 50% while 33% showed positive test 16.70% remained negative. On day 21

**Table 4:** *Comparison of piperacillin (P) group for urine related tests at day-0, day-11 and day-21.*

| | **Day-0** | **Day-11** | **Day-11** | **Day-21** | **Day-0** | **Day-21** |
|---|---|---|---|---|---|---|
| **Urinary Output in 24 Hours (ml)** | 19.17 ±3.87 | 19.17 ±2.07 | 19.17 ±2.07 | 29.17 ±7.84 | 19.17 ±3.87 | 29.17 ±7.84 |
| | P-value =0.50 | | P-value =0.17 | | P-value = 0.20 | |
| **Creatinine Clearance (ml/min)** | 0.81 ±0.17 | 0.70 ±0.13 | 0.70 ±0.13 | 1.47 ±0.51 | 0.81 ±0.17 | 1.47 ±0.51 |
| | P-value = 0.35 | | P-value = 0.11 | | P-value = 0.15 | |
| **Specific Gravity** | 1.002 ±0.001 | 1.005 ±0.001 | 1.005 ±0.001 | 1.005 ±0.001 | 1.002 ±0.001 | 1.005 ±0.001 |
| | P-value = 0.09 | | P-value = 0.40 | | P-value = 0.16 | |

Results represent mean and ± standard error of mean

**Table 5:** *Comparison of tobramycin + piperacillin (TP) groups for blood related tests at day-0, day-11 and day-21.*

| | **Day-0** | **Day-11** | **Day-11** | **Day-21** | **Day-0** | **Day-21** |
|---|---|---|---|---|---|---|
| **BUN (mg/dl)** | 20.99 ±1.30 | 25.42 ±1.31 | 25.42 ±1.31 | 24.22 ±0.92 | 20.99 ±1.30 | 24.22 ±0.92 |
| | P-value < 0.01 | | P-value = 0.18 | | P-value = 0.06 | |
| **Serum Creatinine (mg/dl)** | 1.21 ±0.08 | 1.46 ±0.05 | 1.46 ±0.05 | 1.58 ±0.04 | 1.21 ±0.08 | 1.58 ±0.04 |
| | P-value < 0.01 | | P-value < 0.01 | | P-value < 0.01 | |
| **Serum Sodium (mEq/l)** | 133.00 ±2.11 | 129.00 ±0.86 | 129.00 ±0.86 | 132.00 ±2.88 | 133.00 ±2.11 | 132.00 ±2.88 |
| | P-value < 0.04 | | P-value < 0.20 | | P-value = 0.39 | |
| **Serum Potassium (mEq/l)** | 4.18 ±0.13 | 3.45 ±0.10 | 3.45 ±0.10 | 3.82 ±0.05 | 4.18 ±0.13 | 3.82 ±0.05 |
| | P-value < 0.01 | | P-value < 0.01 | | P-value < 0.01 | |

Results represent mean and ± standard error of mean

**Table 6:** *Comparison of tobramycin + piperacillin (TP) group for urine related tests at day-0, day-11 and day-21*

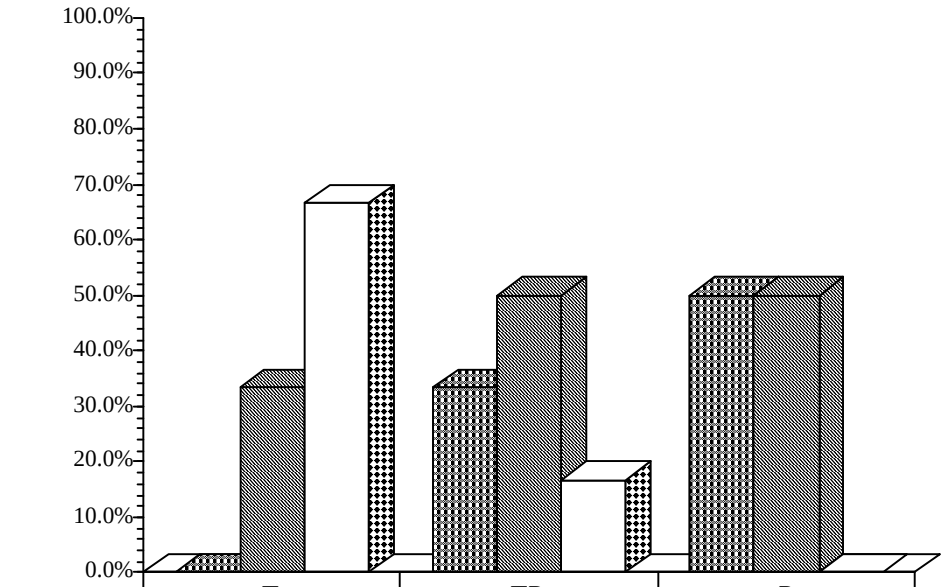
| | **Day-0** | **Day-11** | **Day-11** | **Day-21** | **Day-0** | **Day-21** |
|---|---|---|---|---|---|---|
| **Urinary Output in 24 Hours (ml)** | 19.17 ±2.48 | 21.33 ±2.16 | 21.33 ±2.16 | 26.33 ±2.81 | 19.17 ±2.48 | 26.33 ±2.81 |
| | P-value = 0.26 | | P-value = 0.14 | | P-value < 0.02 | |
| **Creatinine Clearance (ml/min)** | 1.95 ±0.47 | 0.96 ±0.22 | 0.96 ±0.22 | 1.77 ±0.33 | 1.95 ±0.47 | 1.77 ±0.33 |
| | P-value < 0.03 | | P-value < 0.03 | | P-value = 0.24 | |
| **Specific Gravity** | 1.005 ±0.001 | 1.003 ±0.002 | 1.003 ±0.002 | 1.004 ±0.002 | 1.005 ±0.001 | 1.004 ±0.002 |
| | P-value = 0.27 | | P-value = 0.31 | | P-value = 0.35 | |

Results represent mean and ± standard error of mean

66.7% showed positive test for urinary albumin while 33% showed traces. (fig. 1 and 2).

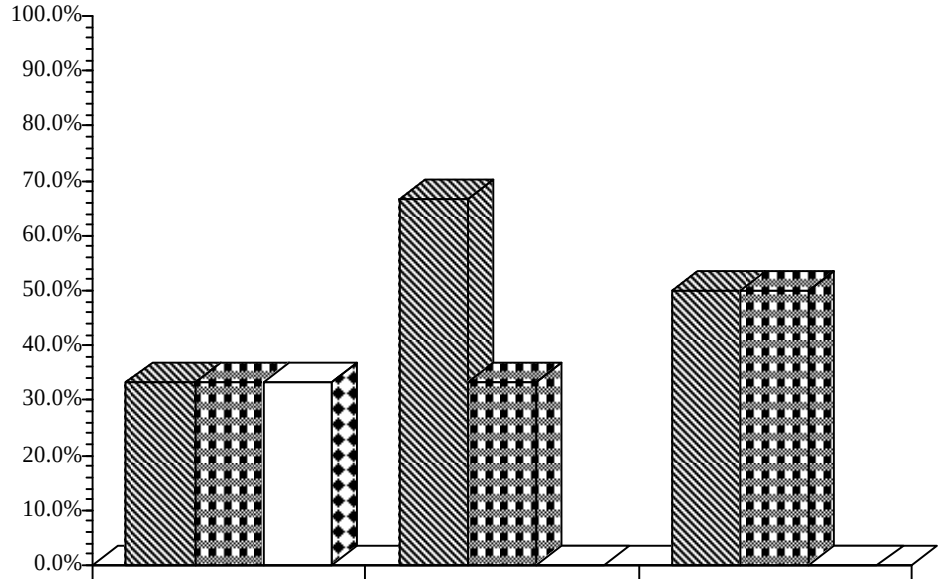
## DISCUSSION

In the present study animals treated with tobramycin showed evidence of mild nephrotoxicity as indicated by modest but significant rise in BUN and serum creatinine and decrease in creatinine clearance. These results are in agreement with those of Cooper et al[12]. In a study carried out by Albarellos et al[13] on six male adult dogs treated with once daily gentamicin, increase in serum creatinine and BUN was seen in one dog while one showed decrease in specific gravity of urine and granular casts were observed in two dogs. They concluded that Gram -ve infection can be treated




| | T | TP | P |
|---|---|---|---|
| Positive | 0.00% | 33.30% | 50.00% |
| Trace | 33.30% | 50.00% | 50.00% |
| Negative | 66.70% | 16.70% | 0.00% |

**Fig 1:** Urinary albumin in various study group at day-11.




| | T | TP | P |
|---|---|---|---|
| Positive | 33.3% | 66.7% | 50.0% |
| Trace | 33.3% | 33.3% | 50.0% |
| Negative | 33.3% | 0.0% | 0.0% |

**Fig 2:** Urinary albumin in various study group at day-21.

with once daily gentamicin administration with little nephrotoxicity.

In the current study significant lower levels of serum potassium ($p < 0.01$) were observed in tobramycin, piperacillin, and tobramycin plus piperacillin treated animals. These results are in agreement with findings of Cronin et al [14] and Brinker et al[15]. They demonstrated that aminoglycoside induced hypokalaemia is secondary to increased renal potassium wasting.

Results of present study did not show any significant nephrotoxicity when piperacillin was used alone. Results are in agreement with those of Hayashi et al[16] who conducted a study on dogs with piperacillin alone and piperacillin-tazobactam combination and noted no significant change in haematological, biochemical, and urine analysis evaluation.

In the present study when tobramycin was used with piperacillin some renal function parameters did change but did not reach toxic levels. Results of a study carried out by Sabra and Branch[17] suggest that salt depletion potentiates aminoglycoside toxicity while salt replacement attenuates it. They demonstrated protective effect of ticarcillin against tobramycin toxicity and thought it to be due to sodium load associated with ticarcillin.

It was **concluded** from the present study that although use of tobramycin results in mild renal toxicity, concurrent use of piperacillin did not augment the renal toxicity and both drugs can be used in combination safely.

**REFERENCES**

1. Chambers HF, Sande MA. Antimicrobial agents: The aminoglycosides. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gillman AG (eds). The Pharmacological Basis of Therapeutics. 9th Ed. New York: 1996; 1103-22.
2. Jawetz E. Aminoglycosides and polymixins. In: Katzung BG (ed). Basic and Clinical Pharmacology. Connecticut: Appleton and Lange, 1995; 680-99.
3. Giannone R, Ferrari E, Panizzardi G, Polio M. Piperacillin in prevention and therapy in gynaecological surgery. Minerva Ginecol 1995; 47: 335-39.
4. Shyr YM, Lui WY, Su CH, Wang LS, Lio CY. Piperacillin/tazobactam in comparison with clindamycin plus gentamicin in the treatment of intra-abdominal infections. Chung Hua I Hsuch Isa Chih Taipei 1995; 56: 102-8.
5. Marie JP, Vekhoff A, Cony MP. Piperacillin/ tazobactam combination + amikacin versus ceftazidime + amikacin in patients with neutropenia and fever. An open multicenter study. Presse Med 1995; 24: 397-401.
6. Chatzinikolaou I, Abi-Said D, Bodey GP. Recent experience with Pseudomonas aeruginosa bacterimia in patients with cancer. Arch Intern Med

2000; 160: 510-9.
7. Tan JS, File MT Jr. Antipseudomonal penicillins. Med Clin North Am 1995; 79: 679-93.
8. Chambers HF. B lactam antibiotics and other inhibitors of cell wall synthesis. In: Katzung BG (ed). Basic and Clinical Pharmacology. 9th Ed. Philadelphia: McGraw Hill, 2003; 734-753.
9. Appel GB, Neu HC. The nephrotoxocity of antimicrobial agents. N Engl J Med 1977; 13: 722-28.
10. Browning MC, Tune BM. Toxicology of B-lactam antibiotics. In: Demain AL, Solomon NA (eds). Handbook of Experimental Pharmacology. Berlin: Springer-Verlag, 1983; 67: 372-97.
11. Stockley IH. Drug interactions. 2nd Ed. Oxford: Blackwell Scientific Publications, 1991; 100-143.
12. Cooper BW, Greger RJ, Soegiarso W. Renal dysfunction during high dose cisplatin therapy and autologous hematopoietic stern cell transplantation: Effect of aminoglycoside therapy. Am J Med 1993; 94: 497-504.
13. Albarellos G, Montoya L, Ambros L, Kreil V, Hallo R, Rebuelto M. Multiple once daily dose pharmakokinetics and renal safety of gentamicin in dogs. J Vet Pharmacol Ther 2004; 27: 21-25.
14. Cronin RE, Bulger RE, Henrich WL. Natural history of aminoglycoside nephrotoxicity in the dog. J Lab Clin Med 1980; 95: 463-74.
15. Brinker KR, Bulger RE, Dobyan DC. Effect of potassium depletion on gentamicin nephrotoxicity. J Lab Clin Med 1981; 98: 292-301.
16. Hayashi T, Watanabe Y, Kumano K, Kitayama R, Yasuda T, Saikawa I, Katahira J, Kumada T, Shimizu K. Protective effect of piperacillin against nephrotoxicity of cephaloridine and gentamicin in animals. Antimicrob Agents Chemother 1988; 32: 912-18.
17. Sabra R, Branch RA. Role of sodium in protection by extended, spectrum penicillins against tobramycin induced nephrotoxicity. Antimicrob Agents Chemother 1990; 34: 1020-25.