International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.4, pp 2149-2154, Oct-Dec 2011

Comparative evaluation of the toxicity of Amikacin and Cefepime on rat's kidney and liver

Bhanu Prajapati*, Manmohan Singhal

School of Pharmaceutical Sciences, Jaipur National University, Jaipur, India.

***Corres.author: prajapati.bhanu23@gmail.com**
Tel : 0141-2779016, Fax: 0141-2754814

Abstract: Aminoglycosides are known to cause oxidative stress related toxicities and tissue injuries and Cefepime is cephalosporin class of antibiotics having potential of free radical scavenging properties. The objective of present study was to evaluate effect of Amikacin on various antioxidant enzymes (Superoxide dismutase Catalase and Glutathione Reductase) along with malonaldialdehyde levels and extracellular antioxidants (Creatinine, Total Bilirubin and uric acid) in renal tissues of male albino rats and to compare the same with the changes produced by Cefepime. Our findings showed that the activities of the antioxidant enzymes were significantly lowered with increase in MDA levels and extracellular antioxidants after single treatment of amikacin as compared to control group. A significant improvement in antioxidant enzymes along with significant lesser decrease in creatinine, total bilrubin, uric acid and MDA levels were observed in Cefepime treated groups as compared to amikacin treated group. These results indicate that, as documented earlier about the broader spectrum of activity of cephalosporins, they also contains the free radical scavenging property and prevents to a greater extent from the hepatotoxicity and nephrotoxicity as compared to the same induced by aminoglycosides.

Key Words:-Amikacin, Cefepime, SGOT, SGPT, Malonaldialdehyde, Total Bilirubin, Creatinine, Cephalosporins, Aminoglycosides, Free radicals, Antioxidant enzymes.

Introduction

Aminoglycosides (amikacin and tobramycin) are most important drugs in clinical use and also essential for the treatment of severe infections caused by gram-negative bacteria. These antibiotics are reported to cause nephrotoxicity, hepatotoxicity, ototoxicity and neuromuscular blocks [1, 2, 3]. Neuromuscular blocks are rare, ototoxicity ranges from 0-62%, hepatotoxicity 0-30% and nephrotoxicity varies from 0-19% [4]. The binding of aminoglycosides in vivo as well as in vitro with negatively charged membranes is associated with impairment of phospholipid catabolism, change in membrane permeability and membrane aggregation. The adverse effect of aminoglycosides has been attributed to the development of an array of alterations in proximal tubule epithelium followed by its destruction, thereby causing kidney dysfunction [5]. Aminoglycosides administration is also reported to induce apoptosis [6], free radical generation [7] and another major adverse effect [1]. Free radicals also play an important role in drug-induced damage to the kidney failure and other organs [8].

Cefepime is a drug from the fourth generation of cephalosporin class of antibiotics having potential of free radical scavenging properties [9]. The purpose of present study was to evaluate effect of aminoglycosides on renal and hepatic tissues, oxidative stress parameters in male albino rats and comparing the same with that of free radical scavenging potential of cephalosporins.

Materials and Methods

Materials

Chemicals

All of the chemicals to be used in the present study have been procured from Sigma, St. Louis, MO, USA

and the marketed preparation of aminoglycoside (amikacin) has been taken for experiment.

Animals and Treatments

Eighteen healthy male albino rats, weighing 150-200 gms were used in the experiment. The rats were fed standard pellet diet and distilled water ad libitum. The rats were divided into 3 groups of six rats each as given below-

- Control Group (isotonic saline treated).
- Amikacin treated group (7.14 mg/kg body weight/day).
- Cefepime treated group (28.5 mg/kg body weight/day).

The respective drugs were administered intramuscularly for 28 days. At the end of treatment, 1ml blood sample were drawn in sodium citracised vials from the heart by cardiac puncture under the light anaesthesia. Blood sample were then centrifuged at 3500 rpm for 4 minutes in order to separate the plasma from blood cells. Sample were then stored at 0-4 ^{o} C before performing the enzyme assay.

Biochemical Assay

Superoxide Dismutase (SOD)

SOD activity was determined by the Method of Fradovich and Misra [10]. The reaction mixture consisted of 1.0ml carbonate buffer (0.2M, pH 10.2), 0.8 ml KCl (0.015M), 0.1ml of blood and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025M). The change in absorbance was recorded at 480 nm at 15 second interval for one minute at 25^{0}C. Suitable control lacking enzyme preparation was run simultaneously. One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

Catalase

Catalase activity was measured by the method of Luck [11]. The reaction mixture consisted of 0.3ml phosphate buffer, (0.2M pH6.8), 0.1ml H_2O_2 (1M) and water to make the final volume to 3.0ml.The reaction was started by adding the suitable aliquot of enzyme preparation. The change in the absorbance was recorded at 15 sec. interval for one minute at 240nm at 25^{0}C. Suitable control was run simultaneously. One Unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from H_2O_2 in 100 sec at 25^{0}C.

Measurement of Lipid Peroxidation

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldialdehyde (MDA) formed, essentially according to Ohkawa et al.[12]. MDA is the most abundant individual aldehyde resulting from lipid peroxidation. It was determined by thio barbituric reaction. The reaction mixture consisted of 100 µl of enzyme preparation, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and water to make up the volume to 4.0 ml. The tubes were boiled in water bath at 95^{0}C for one hour, immediately cooled under running tap water and 1.0 ml of chilled water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) was added and vortexed. The tubes were centrifuged at 3500 rpm for 30 minutes. The upper layer was aspirated out and optical density was measured at 532 nm. The reference standard used was 1,1, 3,3,tetra ethoxy propane. Creatinine levels were determined by the alkaline picrate method using diagnostic kits (Bayer Diagnostics India Ltd.,Baroda, Gujrat India).

Glutathione Reductase Assay

Glutathione reductase activity was measured by the method of Carlberg and Mannervik [13]. The reaction mixture consisted of 1.5ml of potassium phosphate buffer (0.2 M, pH7.0)containing 2mM EDTA, 0.15 ml of 2mM NAPDH, 0.2ml of 20mM oxidised glutathione and added distilled water to make up the final volume to 3.0ml. The reaction was started by adding the 0.1ml of homogenate in the enzyme linearity range. The absorbance was measured at 340nm for one minute at 15 sec. intervals. Control lacking enzyme was run simultaneously. One unit of GR activity is expressed as the amount of NADP formed in one minute by one ml of enzyme prepartion. Calculation of the enzyme activity has been done by using the molar extinction coefficient of NADPH as 6.22 x 10^{3}.

Estimation of SGOT, SGPT, Total Bilirubin, Uric Acid, Creatinine Levels

SGOT, SGPT, Total Bilirubin, Uric Acid, Creatinine levels were determined by using commercially available standard diagnostic kits (Erba diagnostic Mannheim, Germany).

Preparation of Homogenate

Kidney homogenates (15%w/v) were prepared in phosphate buffer-KCl solution containing 0.15mol/L KCl in 0.05mol/L Na2HPO4-NaH2PO4 buffer, pH 6.8. The homogenate was left for at least one hour at 0-4 ^{0}C.

Histopathological Procedure

All rats were sacrificed on the day after the last day of dosing. Cardiac blood samples were taken immediately, after which both kidneys along with liver

were removed and examined grossly. Capsules were striped carefully of each rat and specimen was immersed in 10% neutral buffered formalin for overnight fixation. The fixed samples were dehydrated in series of ethanol of various categories according to their concentration in ascending order and cleared in methyl benzoate. They were then embedded in paraffin and blocks were prepared according to the standard procedure for histopathological evaluation. Tissue sections by the help of microtome (5-6 micron each) were stained with haematoxylin and eosin, and the resulting tissue slides were randomized, masked and examined by a single pathologist without knowledge of the animal's treatment. Same was also done with the liver tissue. The slides were studied in Histopathology section of the department by ordinary light microscope. The tissue sections were examined for inflammatory cell infiltration, single cell and piecemeal necrosis, enlarged swollen hepatic and renal cells with granular cytoplasmic characteristics and vascular abnormalities. Also examined for veno-occlusive changes in liver and for glomerular, tubular and interstitial lesions in kidney of treated animals Histological findings in experimental animals were compared with control group [14, 15].

Statistical Analysis

All values are to be expressed in mean ± S.E.M.. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test is to be applied in order to determined statistical difference between control and experimental groups.

Result

In the present study toxic effect of Amikacin on rat liver and kidney was observed.

The results of the present study show a significant decrease ($p<0.001$, 40.64%) in Catalase, Glutathione Reductase ($p<0.001$, 51.97%), SOD ($p<0.001$, 69.13%) activity along with the increase in the level of various biochemical parameters such as MDA ($p<0.001$, 33.38%), Creatinine ($p<0.01$, 47.36 %), Uric Acid ($p<0.001$, 37.03 %) Total Bilirubin ($p<0.001$, 23.82 %), SGPT ($p<0.001$, 208.88%), SGOT ($p<0.001$, 125.58%) in Amikacin treated groups as compared to that in the control group, whereas in case of Cefepime treated group, there were a significant beneficial changes in the levels of various enzymes such as Catalase ($p<0.001$, 29.94%), GR ($p<0.001$, 25.65%) and other biochemical parameters like MDA ($p<0.001$, 17.37%), Creatinine ($p<0.01$, 44.41 %), Uric Acid ($p<0.01$, 23.45 %), SGPT ($p<0.001$, 44.44%), SGOT ($p<0.001$, 27.90%) on the beneficial side as compared to control competing with that of Amikacin. Exceptions were observed in the result showing the level of SOD ($p<0.001$, 74.07 %) and that of Total Bilirubin ($p<0.001$, 35.14 %).

Amikacin and Cefepime has caused significant change in various biological parameters which is been shown in the table given below.

PARAMETERS EVALUATED	CONTROL	AMIKACIN	CEFEPIME
Catalase	187 ± 1.932	111 ± 2.324 [a] **(-40.64%)**	131 ± 1.592 [a] **(-29.94%)**
GR	1.52 ± 0.015	0.73 ± 0.017 [a] **(-51.97%)**	0.91 ± 0.011 [a] **(-25.65%)**
MDA	656.43 ± 2.173	875 ± 2.781 [a] **(+33.38%)**	770 ± 0.802 [a] **(+17.37%)**
SOD	810 ± 2.033	250 ± 2.933 [a] **(-69.13%)**	210 ± 3.706 [a] **(-74.07%)**
Uric Acid	2.7 ± 0.1673	3.7 ± 0.1653 [a] **(+37.03 %)**	3.33± 0.0881 [b] **(+23.45%)**
Creatinine	0.95± 0.1317	1.45± 0.1668 [b] **(+47.36 %)**	1.37 ± 0.1513 [b] **(+44.41 %)**
Total Bilirubin	0.512 ± 0.0015	0.634±0.0015 [a] **(+23.82 %)**	0.785 ± 0.0020 [a] **(+35.14 %)**
SGPT	45±1.880	139±2.280 [a] **(+208.88%)**	76±2.352 [a] **(+44.44%)**
SGOT	43±1.317	97±2.595 [a] **(+125.58%)**	55±1.880 [a] **(+27.90%)**

Table . Values are expressed in Mean (±) SEM for 6 rats in each group where [a] $p< 0.001$, [b] $p<0.01$ were regarded as significant as compared to control. Values in the parenthesis indicates the percent change in various biochemical parameters to control.

Effect on various Biochemical Parameters

Histopathological Parameters

Light micrograph of the Renal Cortex from the Amikacin treated rats has shown local areas of quite severe coagulative tubular injury associated with glomerulus with a thickened Bowman's capsule and a small area of edema, whereas in the same of that from the control rats, no evidence of tubular and glomerular lesions has been observed. The slides of the micro section from the liver of control has shown normal structure, central vein, normal arrangement of hepatic cords, normal blood sinusoids and hepatocytes which totally varies with that from the amikacin treated rats showing dilated blood sinusoids with severe haemorrhage in the central vein along with various evidences of severe necrosis.

On the other hand, in case of liver section from Cefepime treated rats has shown dilated blood sinosuids with comparatively lesser necrotic view and lesser damage to central vein. Also the cortical section has shown slightly lesser tubular necrosis and comparatively lesser sweeling in glomerulus as compared to that treated with Amikacin.

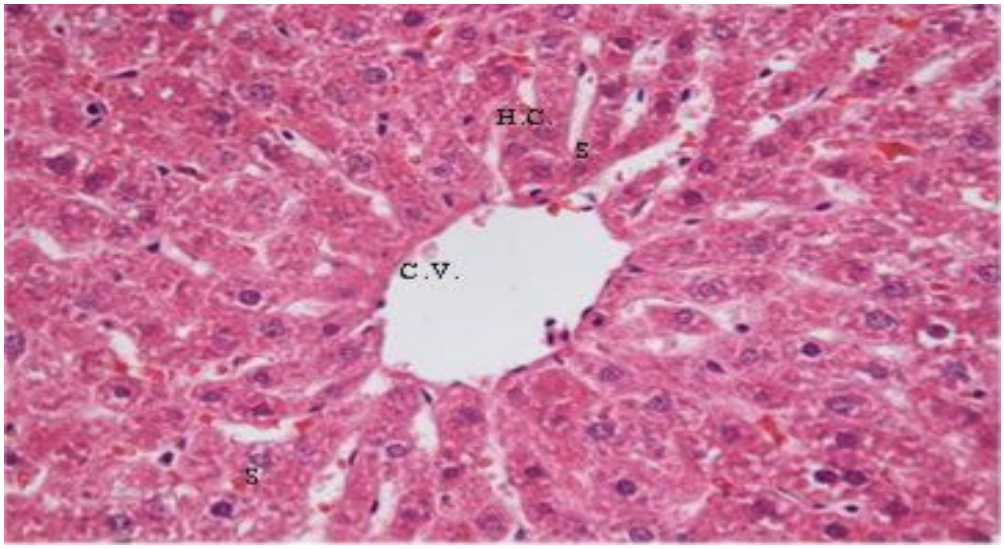

Fig 1. Liver tissue of Control showing normal structure, central vein (C.V.), normal arrangement of hepatic cords (H.C.), normal blood sinusoids (S) and hepatocytes. HE, X 400.

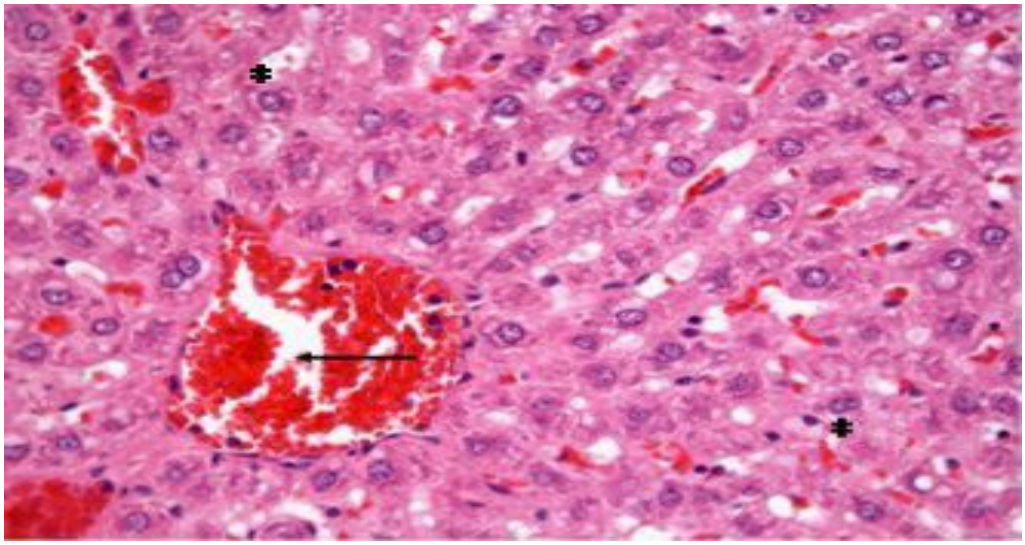

Fig 2. Liver tissue of Amikacin treated rats showing haemorrhage in the central vein (-) with dilated blood sinusoids and necrosis. HE, X 400.

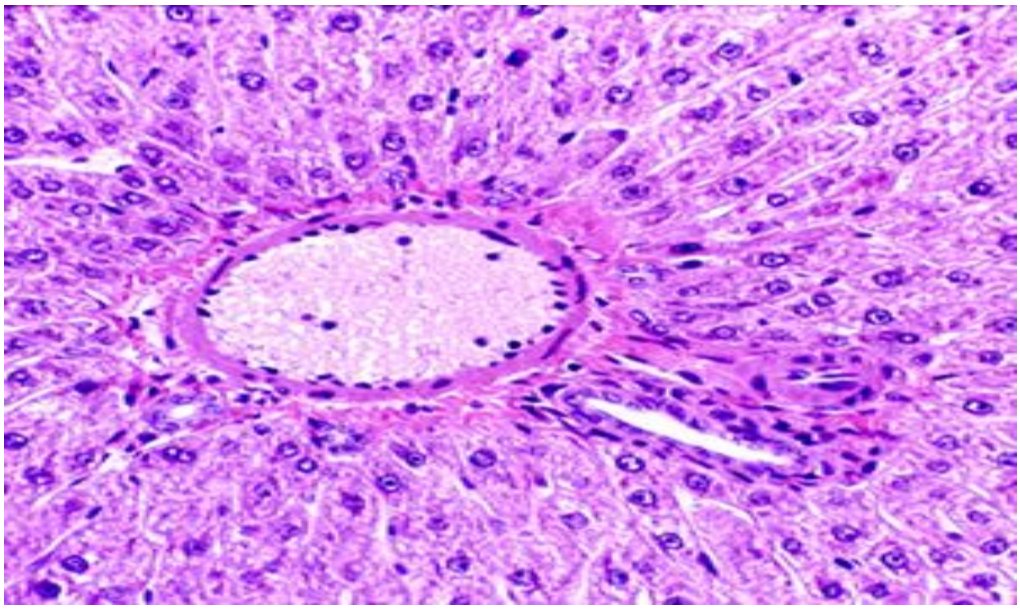

Fig 3. Liver tissue of Cefepime treated rat showing dilated with comparatively lesser necrotic view and lesser damage to central vein as compared to that treated with Amikacin. HE, X 400.

Discussion

Free radical generation causes the tissue injury in the form of nephrotoxicity and ototoxicity due to induction of aminoglycosides. Several studies have been reported that tobramycin and amikacin cause the nephrotoxicity, ototoxicity and alterations in cochlear antioxidant enzyme activities [7,16, 17]. Due to induction of aminoglycosides, the ratio of free radical generating and free radical scavenging enzymes may be disturbed and leading to disrupt signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids. The binding of these aminoglycosides with cellular membrane causes impairment of phospholipid catabolism, changes in membrane aggregation [18] and reduce the activities of phospholipases [19,20].

On other sides, cephalosporins, class of antibiotics such as Cefepime has free radical scavenging potential. Cefepime has low in vitro affinity for the major chromosomally mediated lactamases and good stability against enzymatic hydrolysis [21]. There are several reports to suggest that cephalosporins protect against HOCl-driven oxidative injury. This defense is a consequence of a direct drug scavenging capacity towards HOCl [22].

The present finding demonstrate that treatment with amikacin significantly lowered the antioxidant enzymes activities (catalase, glutathione reductase) along with increased free radical mediated damage (as evidenced by enhanced MDA levels) as well as some extracellular antioxidants (Creatinine, Uric acid and Total bilirubin) in treated mice.

Histological reports gives the evidence that cortical part of the kidney treated with Amikacin showing coagulative necrosis, thickened bowman's capsule and glomerulus, small area of oedema along with tubular degeneration, hypertrophy of epithelial cells. Severe haemorrhage in central vein of liver section with dilated blood sinusoids along with the hepatic tissue necrosis has also been observed, which clearly indicates the oxidative tissue injury by the amikacin to the hepatic and nephrotic environment.

Cortical areas of the samples of the rats treated with Cefepime has shown comparatively slight local areas of tubular necrosis and a slight swelling in glomerulus. Liver section has shown dilated blood sinosuids with comparatively lesser necrotic view and lesser damage to central vein as compared to that treated with Amikacin. Similar finding was reported with other aminoglycosides such as streptomycin and gentamycin in kidney and heart [23].

Conclusion

These biological parameters indicate that administration of amikacin causes the renal and hepatic toxicity. On the other hand Cefepime which has proven its capability of scavenging the free radical damage is found to be a better option to treat infection due to its broader spectrum of activity.

References

1. Gilbert DN., Mandell G. L., Bennett J. E., Dolin R.., Aminoglycosides In Principles and Practice of Infectious Diseases, 2000, 5, 307–336.
2. Tulkens P M., Nephrotoxicity of amino glycosides, Toxicol Lett, 1989; 46: 107–23.
3. Cipullo JP., Burdmann EP., Aminoglycoside nephrotoxicity, Braz J cardiovasc Sur, 2006, 21(4): 444-52.
4. Mingeot-Leclercq MP., Glupczynski Y., Tulkens P M., Aminoglycosides:activity and resistance, Antimicrob. Agents Chemother., 1999, 43, 727–37.
5. Lang H., Liu C., Apoptosis and hair-cell degeneration in the vestibular sensory epithelia of the guinea pig following a gentamicin insult, Hear Res, 1997, 111, 177–84.
6. Klemens JJ,, Meech RP., Hughes LF., Somani S., Campbell KCM, Antioxidant enzyme levels inversely Covary with hearing loss after amikacin treatment, J Am Acad Audiol, 2003, 14 (4), 134-42.
7. Leclercq MPM., Tulkens PM., Aminoglycosides: Nephrotoxicity, Antimicrob Agents Chemother, 1999, 43 (5), 1003-12.
8. Cantin A., Woods DE., Protection by antibiotics against Myeloperoxidase-dependant cytotoxicity to lung epithelial cells in vitro, J Clin Invest, 1993, 91, 38-45.
9. Misra HP, Fridovich I., The role of superoxide anion in the auto-oxidation of epinephrine and a sample assay for Super-oxide dismutase, J Biol Chem, 1972, 247, 3170-75.
10. Luck. Catalase, Method in Enzymatic analysis, NY: Academic Press, 1965, 885-94.
11. Carlberg I., and Mannervik, B., Method. in Enzymology, 1985, 113, 485-90.
12. Ohkawa H., Ohishi N., Yagi K., Assay of lipid per-oxidation in animal tissue by thio barbutric acid reaction, Anal Biochem, 1979, 95, 351-58.
13. Gilbert DN., Mandell G. L., Bennett J. E., Dolin R.., Aminoglycosides In Principles and Practice of Infectious Diseases, 1995, 4, 279-306.
14. Ozturk HS., Kavutcu M., Kaçmaz M., Canbolat O., Durak I., The effects of gentamicin on the activities of glutathione peroxidase and superoxide dismutase enzymes and malondialdehyde levels in heart tissues of guinea pigs, Cur Med Res Opin, 1997, 14, 47-52.
15. Afshar S., Farshid A., Heidari R., Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion, Toxicol, Ind. Health, 2008, 24 (9), 581-586.
16. Rybak LP., Somani S., Ototoxicity : Amelioration by Protective Agents, Annl New York Acad Sci, 1999, 884, 143-51.
17. Vijayalekshmy KS., Menon V., Leelamma S., Role of antibiotics in lipid peroxidation, Ind J Biochem Biophys, 1992, 29, 371–74.
18. Van Bambeke FPM., Tulkens RB., Mingeot-Leclercq MP., Aminoglycoside antibiotics induce aggregation but not fusion of negatively-charged liposomes, Eur J Pharmacol, 1995, 289, 321–33.
19. Hostetler KY., Hall LB., Aminoglycoside antibiotics inhibit lysosomal phospholipase A and C from rat liver in vitro, Biochim Biophys Acta, 1982, 710, 506–9.
20. Laurent G., Carlier MB., Rollman B., Van Hoof F., Tulkens P., Mechanism of aminoglycoside-induced lysosomal phospholipidosis: in vitro and in vivo studies with gentamicin and amikacin, Biochem Pharmacol, 1982, 31, 3861–70.
21. Soejima A., Ishizuka S., Miyake N., Fukuoka K., Suzuki M., Kamiya Y., Simultaneous inhibition of renal phospholipase A(2) and glutathione synthesis by manoalide and DLbuthionine sulfoximine induces acute tubular dysfunction in rats, Exp Nephrol, 2000, 8 (2), 84-90.
22. Lapenna D., Cellini L., Gioia DS., Mezzetti A., Ciofani G., Festi D., Cuccurullo F., Cephalosporins are scavengers of hypochlorous acid, Biochem Pharmacol, 1995, 49 (9), 1249-54.

*****