Journal of Animal and Veterinary Advances 7 (9): 1038-1041, 2008
ISSN: 1680-5593

Histopathological Study of Vitamins A and C Effects on the Reduction of Gentamycin Nephrotoxicity in Rats

[1]N. Hadjipour, [2]S.M. Naghib and [3]M. Davanian
[1]Department of Pathological Sciences, School of Veterinary Medicine,
[2]Young Researchers Club, [3]Department of Veterinary Medicine,
Islamic Azad University, Kazeroon Branch, Kazeroon, Iran

Abstract: Gentamicin (GM) is widely used as a bactericidal agent for treatment of severe gram negative infections. However, the clinical use of gentamicin is limited due to its known nephrotoxic effects. Previous studies were done on other antioxidant vitamins and in this study we directly compare the effects of Vitamins A and C on reduction of gentamycin nephrotoxicity on rat kidney. Thirty adult male Sprague-Dawley rats were randomly assigned to 5 groups: group 1(control) was injected with strile normal saline; group 2 (Gm 5-8 mg kg^{-1} IM); group 3(GM +VitA); group 4(GM + VitC); group 5(GM + VitA + VitC). The injection courses for all groups were 14 days. Then histopathologic slides were provided from these kidneys. In slides observations by light microscope, 4 levels of necrosis were defined (the level-making method will be mentioned in full text): 1 = light necrosis, 2 = medium necrosis, 3 = severe necrosis and 4 = extra severe necrosis. The 6 rats in second group (injected by Gentamycin) showed levels 3,4,4,4,3,4 of necrosis in kidney (average:3/66). Co-injecting of Vit. A with Gentamycin had a trivial effect on nephrotoxicity reduction, so that the necrosis levels in the rats of this group were 4,3,3,3,3,4 (average:3/3). The rats injected by GM + VIT.C showed a remarkable effect on the reduction of Gentamycin nephrotoxicity by causing 1,2,2,1,1,1 levels of necrosis (average: 1/33). The last group (co-injecting of GM, VIT.A and VIT.C) showed a average of 2/5 in necrosis level (levels of 3,3,2,3,2,2). So by statistical analysis it can be concluded that combination of Vit. C with Gentamycin administration can be the drug of choice in infection treatments to reduce Gentamycin nephrotoxicity.

Key words: Histopatological, gentamycin, nephrotoxicity, Vitamins A and C

INTRODUCTION

Aminoglycosides are bactericidal antibiotics that interfere with protein synthesis by causing misreading of the genetic message and stimulation of faulty production of RNA. Gentamycin is an antibiotic belonging to the aminoglycosides and it is widely used in the treatment of Gram-negative infections (Barza, 1987). However, its nephrotoxic action has limited the extent of its use (Mingeot and Tulkens, 1999). Nephrotoxicity occurs as a result of proximal tubular damage and glomerular dysfunction. It iss because of gentamycin ability to stimulate the generation of reactive oxygen species (ROS) (Cuzzocrea *et al*., 2002). Although, the mechanism of gentamicin-induced cell injury and cell death is still unclear (Cuzzocrea *et al*., 2002). A considerable number of studies have been done on Effects of other antioxidant vitamins on nephrotoxicity of gentamycin and suggesting more effectiveness of combination therapy by cosupplementation of 2 antioxidants (Ademuyiwa *et al*., 1990; Kavutcu *et al*., 1996; Abdel-Naim *et al*., 1999). Recently, it has been shown that both vitamins E and C decreased lipid peroxidation and increase activity of antioxidant enzymes in the kidneys of diabetic rats. Various histological and histopathological studies were undertaken on effects of vitamins on nephrotoxicity of gentamycin in different domestic animals at light microscopic. However, no histopathological evaluation of effects of vitamins A and C on the reduction of Gentamycin nephrotoxicity in rats has been done. Therefore, present study was undertaken.

MATERIALS AND METHODS

Experiment was carried out in thirty male Sprague-Dawley rats weighing 200-300 were housed under

Corresponding Author: N. Hadjipour, Department of Pathological Sciences, School of Veterinary Medicine, Islamic Azad University, Kazeroon Branch, Kazeroon, Iran

controlled environmental condition (27+2°C) and the animals were kept in stress-free condition with free access to water and normal diet.

Animal care was in compliance with the guidelines of the Animal and Human Ethical committee of Kazeroon Medical Sciences University.

Animals were randomly classified in 5 groups:

- Control group.
- GM group: these rats were treated intramuscullary with the GM (5-8 mg kg^{-1}) for 14 days.
- GM+Vit A group: GM and vitamin A were injected once for fourteen days (1-10 mg kg^{-1}).
- GM+vit C, with vitamin C intramuscullary for 14 days (10-30 mg kg^{-1}).
- GM+VIT A+VIT C group: injection for 14 days.

Sample collection and histopathological examination: Rats were anesthetized deeply with ether and both kidnyes were excised for histopathological studies and fixed with 10% bufferred formalin solution then placed in fresh fixative solution and then fixed in 5% bufferd formaline solution to pH 7.3 at room temperature for 1 week. kidneys sagittally were cut and half of each kidney was selected for pathological examination, then processed for paraffin embedding.

A 6 μm-thick sagittal sections from each kidney were taken from each block. sections were stained with Hematoxylin and Eosin.

A pathologist examined all the slides by light microscope.

For each renal section, whole slides were examined for tubular necrosis. slides were examined and assigned for severity of changes and four levels of necrosis were defined. A significant difference between all groups was seen (2, 3, 4, 5) Kruskal-wallis test ($p = 0.001$, $df = 3$, $x^2 = 17.552$). But no significant difference was observed between groups 2 and 3 (Mann-whitney test ($p = 0.269$)).

RESULTS

In this stage, the histopathologic slides were carefully examined by a pathologist. In watching the slides, some grading levels were provided to be applied on every slide. These levels include:

Level 1 (light necrosis): Average of 0-2 tubules necrosis in 10 views of any slide by light microscope.

Level 2 (medium necrosis): Average of 2-5 tubules necrosis in 10 views of any slide by light microscope.

Level 3 (severe necrosis): Average of 5-10 tubules necrosis in 10 views of any slide by light microscope.

Level 4 (extra severe necrosis): Average of more than 10 tubules necrosis in 10 views of any slide by light microscope.

By considering these grades, these results were gained:

- The 6 rats in second group (injected by Gentamycin) showed levels 3, 4, 4, 4, 3, 4 of necrosis in kidneys (average: 3/66). So a necrosis of severe to extra severe was seen in the slides of this group (Fig. 1).
- Co-injecting of Vit. A with Gentamycin had a trivial effect on nephrotoxicity reduction, so that the necrosis levels in the rats of this group were 4, 3, 3, 3, 3, 4 (average: 3/3). So, a severe to extra severe was seen in this group too (Fig. 2).

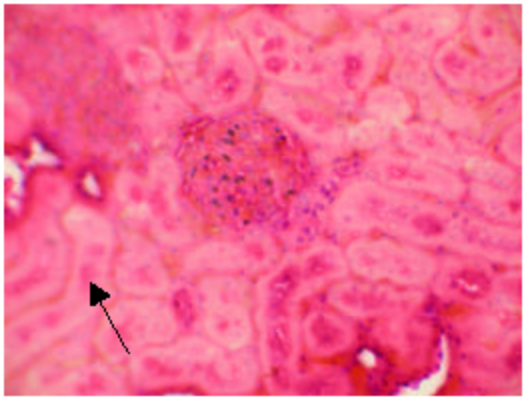

Fig. 1: A kidney section from the group injected by Gentamycin. Extra severe coagulative necrosis is seen in the section. Necrosis rate average 3/66 (400X) (H and E). The arrow shows a typical tubular necrosis

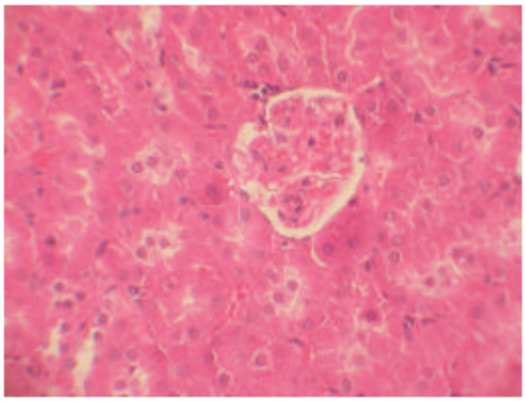

Fig. 2: A kidney section from the group injected by Gentamycin and Vit. A. The rate of necrosis is close to severe. Average rate: 3/3 (400X)(H and E)

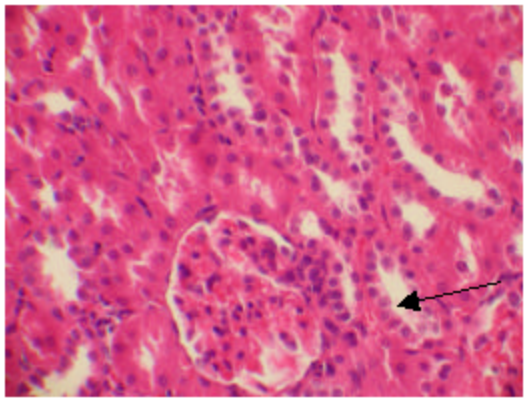

Fig. 3: A kidney section from the group injected be Gentamycin + Vitamin C.. Existence of nucleus in tubule cells shows a light to medium level necrosis. Average rate 1/33 (400X)(H and E). The arrow shows a tubule with cells having nucleus

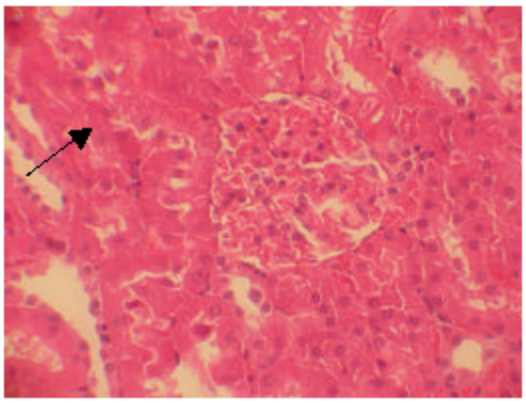

Fig. 4: A Kidney section from the group injected by Gentamycin + Vitamin C + Vitamin A. The medium to severe average rate (2/5) was seen in the section. Nucleusless tubular cells show coagulative necrosis (400X) (H and E)

- The rats injected by GM + VIT.C showed a remarkable effect on the reduction of Gentamycin nephrotoxicity by causing 1, 2, 2, 1, 1, 1 levels of necrosis (average: 1/33). So a necrosis of light to medium was seen in these slides (Fig. 3).
- The last group (co-injecting of GM, VIT.A and VIT.C) showed a average of 2/5 in necrosis level (levels of 3, 3, 2, 3, 2, 2). So, a necrosis of medium to severe was seen in these slides (Fig. 4).

DISCUSSION

Aminoglycoside antibiotics including GM can produce nephrotoxicity in human. Proximal tubular cells are a major site of damage in patients treated with GM or the antibiotic amikacin (Wiland and Szechcinski, 2003). GM binds to the cell wall phospholipids, blocking the chain reactions of phosphatidyl inositol which impairs cell integrity. It has been shown that aminoglycoside antibiotics exert their adverse renal effects by generation of ROS. Formation of ROS following bioactivation of GM has been reported (Sha and Schacht, 1999). Some studies demonstrated that antioxidant administration have ameliorated GM-induced nephropathy (Pedraza-Chaverri *et al*., 2003; Atessahin *et al*., 2003). Co-supplementation of vitamins C and E significantly reduced GM-induced renal toxicity (Kadkhodaee *et al*., 2005).

In this study by analyzing the results by statistical calculations, it can be stated that injecting Vit. A beside GM has no acceptable effect on reducing the nephrotoxicity of GM.

Although, in microscopic vision, accompanying Vit. A and C with GM showed diminishing of necrotoxicity of GM from severe level to medium level, but in statistical analysis, no significant and acceptable difference was proved. But injecting Vit C beside GM presented a more remarkable and acceptable difference than injecting only GM.

This study revealed that the GM-induced renal toxicity significantly reduced by supplementation of vitamins C but accompanying Vit. A to Gentamycin had a trivial or no influence in reducing the nephrotoxicity.

By comparing this study with other similar studies on different vitamins in reducing the nephrotoxicity of GM, it is recommended to physicians and veterinary doctors to prescribe Vit. C as a complimentary beside Gentamycin. This can lower the side effects of Gentamycin that is a big problem in treating the body infections.

ACKNOWLEDGEMENT

Financial support by the Young Researchers Club of Islamic Azad University of kazeroon branch is greatly appreciated. Further acknowledgements are also given to Mrs. Galledari and Mrs. Rezaea for their technical assistance.

REFERENCES

Abdel-Naim, A.B., M.H. Abdel-Wahab and F.F. Attia, 1999. Protective effects of vitamin E and probucol against gentamicin-induced nephrotoxicity in rats. Pharmacol. Res., 40: 183-187.

Ademuyiwa, O., E.O. Ngaha and F.O. Ubah, 1990. Vitamin E and selenium in gentamicin nephrotoxicity. Hum. Exp. Toxicol., 9 (5): 281-288.

Cuzzocrea, S., E. Mazzon, L. Dugo, I. Serraino, R. Di Paola and D. Britti *et al*., 2002. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol., 450: 67-76.

Ho, J.L. and M. Barza, 1987. Antimicrob. Agents Chemother., 31: 485-491.

Kadkhodaee, M., H. Khastar, M. Faghihi, R. Ghaznavi and M. Zahmatkesh, 2005. Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. Exp. Physiol., 90 (4): 571-576.

Kadkhodaee, M., H. Khastar, H.A. Arab, R. Ghaznavi, M. Zahmatkesh and M. Mahdavi-Mazdeh, 2007. Antioxidant vitamins preserve superoxide dismutase activities in gentamicin-induced nephrotoxicity. Transplant Proc., 39 (4): 864-865.

Kavutcu, M., O. Canbolat, S. Ozturk, E. Olcay, S. Ulutepe, C. Ekinci, I.H. Gokhun and I. Durak, 1996. Reduced enzymatic antioxidant defense mechanism in kidney tissues from gentamicin-treated guinea pigs: Effects of vitamins E and C. Nephron, 72: 69-74.

Mingeot-Leclercq, M.P. and P.M. Tulkens, 1999. Aminoglycosides: Nephrotoxicity. Antimicrob. Agents Chemother., 43: 1003-1012.

Pedraza-Chverri, D., Britti, A. De Sarro, A.P. Caputi and S. Cuzzocrea, 2003. Effect of N-acetylcysteine on gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol., 424: 75-83.

Sha, S.H. and J. Schacht, 1999. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res., 128: 112-118.

Walker, D., S. Szram, T. Kornatowski, L. Szadujkis-Szadurski, J. Kedziora and G. Bartosz, 2003. Effect of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. Nephron Exp. Nephrol., 95: 134-143.

Wiland, P. and J. Szechinski, 2003. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol. J. Pharmacol., 55: 631-637.