International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.562.566

Research Article
Protective Effect of Alpha-linolenic Acid on Gentamicin Induced Nephrotoxicity in Mice

[1]H.M. Kaplan, [2]V. İzol, [2]İ.A. Arıdoğan, [1]E. Olgan, [1]A.A. Yegani, [3]P. Pazarcı and [1]E. Şingirik

[1]Department of Pharmacology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey
[2]Department of Urology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey
[3]Department of Medical Biology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey

Abstract

Background: Renal tubular cells are exposed to high toxin concentrations more than other tissues because of active tubular secretion, reabsorption and urine concentration mechanisms in kidneys. Due to this reason, renal tubules are direct targets of nephrotoxicity. Alpha-linolenic acid is a carboxylic acid, whose anti-inflammatory and anti-oxidant effects are shown in various studies. Because of this, a study to examine the protective effects of alpha-linolenic acid on nephrotoxicity is planned. **Materials and Methods:** Gentamicin is administered to mice for 9 days to form nephrotoxicity and alpha-linolenic acid is administered to mice for 9 days to evaluate protective effects. Cyclooxygenase-2, phospholipase A2 and inducible nitric oxide synthase enzymes in their kidneys are analyzed by using ELISA method to compare the nephrotoxicity levels. **Results:** Gentamicin administration increased the expression of cyclooxygenase-2, phospholipase A2 and inducible nitric oxide synthase enzymes. Alpha-linolenic acid administration to mice that are administered gentamicin previously decreased the rate of increase of the cyclooxygenase-2 and inducible nitric oxide synthase caused by gentamicin while, it didn't have any effect on phospholipase A2 increase. **Conclusion:** Gentamicin caused an increase in cyclooxygenase-2, phospholipase A2, inducible nitric oxide synthase enzymes in kidneys. Application of alpha-linolenic acid decreased the increase of cyclooxygenase-2 and nitric oxide synthase enzymes significantly while, having no effect on the increase of phospholipase A2. In conclusion, this study shows that the gentamicin administration causes nephrotoxicity and the use of alpha-linolenic acid can be helpful against this toxic effect.

Key words: Nephrotoxicity, alpha-linolenic acid, cyclooxygenase-2, phospholipase A2, inducible nitric oxide synthase

Received: March 23, 2016 Accepted: April 06, 2016 Published: June 15, 2016

Citation: H.M. Kaplan, V. İzol, İ.A. Arıdoğan, E. Olgan, A.A. Yegani, P. Pazarcı and E. Şingirik, 2016. Protective effect of alpha-linolenic acid on gentamicin induced nephrotoxicity in mice. Int. J. Pharmacol., 12: 562-566.

Corresponding Author: H.M. Kaplan, Department Pharmacology, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Saturated fatty acids can accumulate in the body since they are in solid state at room temperature. However, poly unsaturated fatty acids are in liquid state in room temperature and they are also vital for human survival. Due to this reason they are named as fundamental fatty acids and they are separated in to two groups which are n-6 and n-3. Main sources for n-6 fatty acids are corn and soybean oils and main sources for n-3 fatty acids are flaxseed oil, nuts and fish oils. Flaxseed oil and nuts mostly contain alpha-linolenic acid while, fish oil mostly contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The EPA and DHA should be ingested because they can not be synthesized and therefore called essential fatty acids[1].

Alpha-linolenic acid is protective for heart and cardiovascular system[2,3]. It is a precursor for EPA and DHA which joins arachidonate structure[4]. Blood pressure of spontaneous hypertensive rats is shown to decrease which are on a alpha-linolenic acid diet[5]. Alpha-linolenic acid also have antioxidant properties. It reduces oxidative stress which helps to prevent the inflammation[6]. It also reduces the inflammation caused by lipopolysaccharides (LPS). In addition, alpha-linolenic acid inhibits the translocation of nuclear factor kappa-B (NF-κB) and the phosphorylation of mitogen-activated protein kinase (MAPK) which decreases the expression of inflammation factors such as inducible Nitric Oxide Synthase (iNOS), cyclooxygenase (COX)-2 and TNF-alpha[7].

Various studies shown that one of the main reasons for acute intrinsic renal insufficiency is aminoglycoside nephrotoxicity. Nephrotoxicity can be defined as nephrotoxic renal insufficiency. Kidneys are prone to be affected by toxic effects of the drugs and other endogenic and exogenic toxins due to their function in blood perfusion, metabolic activity and excretion. Renal tubular cells are exposed to high toxin concentrations more than other tissues because of active tubular secretion, reabsorption and urine concentration mechanisms in kidneys. Due to this reason, renal tubules are direct targets of nephrotoxicity. Tubular necrosis is the significant feature of the nephrotoxicity. Due to this, it is also called acute tubular necrosis[8].

Aminoglycoside antibiotics are widely used in control and treatment of the Gram (-) aerobic infections. Although, gentamicins have the most widespread application areas among these antibiotics, its usage is limited because of its nephrotoxic effects. Aminoglycoside antibiotics are responsible for nearly 10% of the acute renal insufficiency incidences. Due to this, aminoglycoside antibiotics are used as a model in acute renal insufficiency incidences[8].

Various studies showed that the COX pathway, which functions in inflammation, accompanies gentamicin nephrotoxicity. In a study, selective COX-2 inhibitor is reported to be decreasing the nephrotoxicity caused by gentamicin[9]. Furthermore, gentamicin application is determined to be causing an increase in the activity of phospholipase A2[10]. In another study conducted, nephorotoxicity formed by gentamicin application caused an increase in iNOS enzyme[11].

The aim of this study was to evaluate the effects alpha-linolenic acid, which is shown to have antiinflammatory effects on iNOS, phospholipase A2 and COX-2 enzymes whose levels are increased in nephrons by the gentamicin.

MATERIALS AND METHODS

Male mice (8 weeks old, balb/c, albino) that are obtained from the Experimental Animal Center in Çukurova University, in Adana are used in study. This study was approved by the Animal Care Committee and Ethics Committee of Cukurova University.

Mice are divided into three groups which are: Control group, gentamicin group and alpha-linolenic acid group. To gentamicin group, 100 mg kg^{-1} gentamicin is applied intraperitoneally once a day for 9 days. To alpha-linolenic acid group, 100 mg kg^{-1} gentamicin is applied intraperitoneally together with 70 mg kg^{-1} alpha-linolenic acid which is applied by using a gavage for 9 days. Physiological serum is applied intraperitoneally to control group under same experimental conditions for 9 days. At the end of the protocol described above, cervical dislocation is applied to the mice. Kidneys of the mice are stored in eppendorf tubes at -80°C for later use in quantitative analysis.

Quantitative analysis

Tissue homogenization: Frozen tissue samples that are stored in eppendorf tubes are treated with 3 mL g^{-1} RIPA (Radio-immunoprecipitation assay) buffer, 30 µL PMSF (phenylmethanesulfonyl fluoride), 30 µL sodyum vanadate and 30 µL protease inhibitor. Then, homogenates are obtained by using ultrosonication on those tubes on ice. Homogenates are then centrifuged at 10,000 RPM for 10 min and supernatants are taken and pellets are discarded.

Protein quantification: Bradford method is used to quantify the protein in homogenized tissues. By using Bovine serum albumin (1 μg mL^{-1}), 1, 2, 3, 5, 7, 8, 10 μg mL^{-1} standards are prepared. Then, 10 μL is taken from every sample and completed to 100 μL by adding distilled water. Lastly, 1 mL Bradford solution is added to standards and samples, vortexed and absorbances at 595 nanometer are measured manually. Protein quantification (μg μL^{-1}) is done according to the standart curve drawn in Prism software.

ELISA (Enzyme linked immunosorbent assay) test: The ELISA test is used to examine the expression and activity of COX-2, phospholipase A2 and iNOS enzymes.

Statistic analysis: Results were expressed as Mean±SEM and n refers to the number of animals used for each experiments. Differences in results between tissues were tested by analysis of variance (ANOVA) corrected for multiple comparisons (Bonferroni corrections). The p-values less than 0.05 were considered to be significant.

RESULTS

ELISA COX-2 enzyme quantification: While, gentamicin application caused an increase in the COX-2 enzyme in kidneys, application of alpha-linolenic acid decreased this increase significantly (Fig. 1). Mean values of COX-2 concentrations for control, gentamicin and gentamicin+ alpha-linolenic acid groups are found to be 9499 pg mL^{-1} (SEM 241.2), 11422 pg mL^{-1} (SEM 461.1), 9617 pg mL^{-1} (SEM 107.8), respectively.

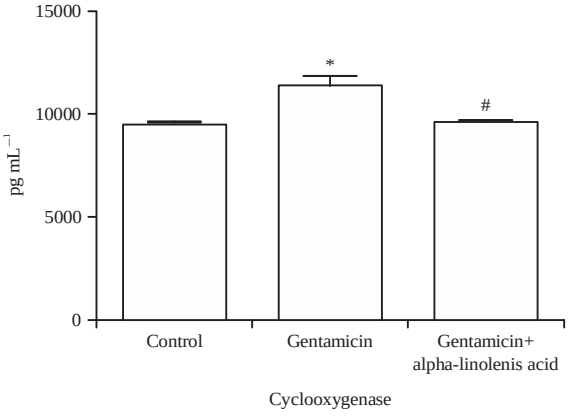

Fig. 1: Effect of alpha-linolenic acid on cyclooxygenase-2 enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA, *post hoc*: Bonferroni, *Control at p<0.05, #Gentamicin at p<0.05

ELISA phospholipase A2 enzyme quantification: While, gentamicin application caused an increase in the phospholipase A2 enzyme in kidneys, application of alpha-linolenic acid had no effect on this increase (Fig. 2). Mean values of phospholipase A2 concentrations for control, gentamicin and gentamicin+alpha-linolenic acid groups are found to be 1766 pg mL^{-1} (SEM 72.9), 2448 pg mL^{-1} (SEM 139.5), 2603 pg mL^{-1} (SEM 112.3), respectively.

ELISA iNOS enzyme quantification: While, gentamicin application caused an increase in the iNOS enzyme in kidneys, application of alpha-linolenic acid decreased this increase significantly (Fig. 3). Mean values of iNOS concentrations for control, gentamicin and gentamicin+alpha-linolenic acid

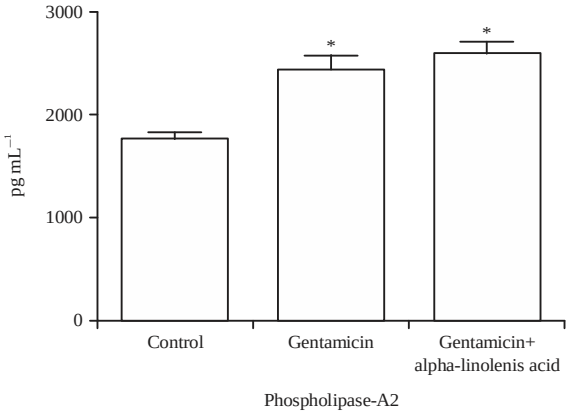

Fig. 2: Effect of alpha-linolenic acid on phospholipase A2 enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA, *post hoc*: Bonferroni, *Control at p<0.05, #Gentamicin at p<0.05

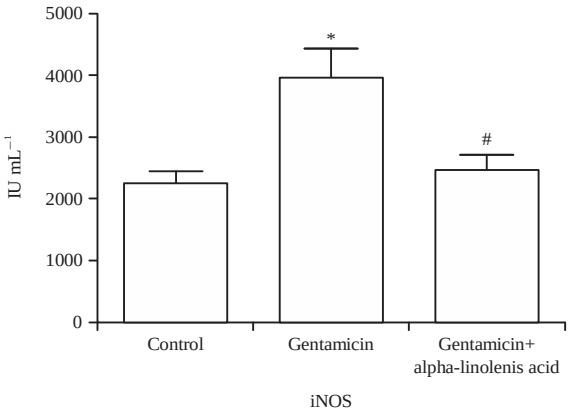

Fig. 3: Effect of alpha-linolenic acid on iNOS enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA, *post hoc*: Bonferroni, *Control at p<0.05, #Gentamicin at p<0.05

groups are found to be 2251 IU mL^{-1} (SEM 191.1), 3964 IU mL^{-1} (SEM 475.5), 2473 IU mL^{-1} (SEM 232.1), respectively.

DISCUSSION

In this study, the enzymes iNOS, phospholipase A2 and COX-2 which functions in inflammatory response as biological mediators is examined. Various studies confirmed the importance of inflammation in nephrotoxicity caused by gentamicin[9-11]. The NO produced by one of these mediators, iNOS have a pathogenic role in acute and chronic inflammatory diseases[12-14]. Studies showed that nephron damage caused by gentamicin is accompanied by iNOS enzyme[11]. The iNOS enzyme is shown to be decreased by the alpha-linolenic acid in previous studies[7]. In addition, increased oxidative stress increases superoxide and peroxynitrite production whose formations are also shown to be reduced by alpha-linolenic acid supplement[15,16]. According to the results of this study, application of alpha-linolenic acid also decreased the increment of iNOS enzyme caused by gentamicin. Alpha-linolenic acid supplements will help to prevent the nephrotoxicity by inhibiting iNOS.

The COX-2 enzyme, which is an inflammatory mediator and prostaglandins synthesized by this enzyme also plays a role in gentamicin nephrotoxicity and there are studies showing that this enzyme increases in nephrons on gentamicin application[4]. Studies showed that selective COX-2 inhibitors reduce the risk of nephrotoxicity caused by gentamicin. At the same time, alpha-linolenic acid is shown to decrease the LPS induced COX-2 enzyme. Results of this study also show similarities to previous studies and alpha-linolenic acid decreased the increment caused by gentamicin in COX-2 enzyme. However, previous studies determined that the increased iNOS activity reduces the COX-2 expression[17]. This also suggests that the reduction in COX-2 may have been caused by the increased iNOS.

Another enzyme examined in this study, phospholipase A2 is also shown to be increased by gentamicin application in various studies[18]. According to the results of this study, application of alpha-linolenic acid couldn't decrease this increment. Studies showed that, gentamicin application induces the oxidative stress which causes an increase in intracellular calcium levels and this increase causes an increase in the activity of phospholipase A2 enzyme as a result[19-21]. Alpha-linolenic acid have antioxidative properties because of double bonds in its structure[6]. While, authors expected to see a decrease in increment of phospholipase A2 caused by gentamicin due to application of antioxidant alpha-linolenic acid, it had no effect according to the results. This can be due to the insufficient effect of antioxidative properties of alpha-linolenic acid in nephrons or gentamicin causing this increment via other mechanisms.

Oxidative stress causes the activation and nuclear translocation of NF-κB which are both key factors in renal inflammation by regulating the gene expression of cytokines and adhesion molecules[22]. Thereby, reduction of oxidative stress by using alpha-linolenic acid can prevent NF-κB translocation at the early stages of inflammation and thus, can prevent the nephron damage by inhibiting the migration of monocytes and macrophages through decreasing the expression of inflammation mediators such as iNOS and COX-2.

CONCLUSION

This study shows that, application of alpha-linolenic acid reduces the inflammatory response due to increased iNOS and COX-2 enzymes and renal damage caused by gentamicin by inhibiting these enzymes. Thus, application of alpha-linolenic acid reduces the nephrotoxicity risk caused by gentamicin.

ACKNOWLEDGMENT

This study was funded by Cukurova University (TSA-2014-2656).

REFERENCES

1. Goodhart, R.S. and M.E. Shils, 1980. Modern Nutrition in Health and Disease. 6th Edn., Lea and Febiger, Philadelphia, PA., ISBN: 9780812106459, Pages: 1370.
2. Bucher, H.C., P. Hengstler, C. Schindler and G. Meier, 2002. N-3 polyunsaturated fatty acids in coronary heart disease: A meta-analysis of randomized controlled trials. Am. J. Med., 112: 298-304.
3. De Lorgeril, M., P. Salen, J.L. Martin, I. Monjaud, J. Delaye and N. Mamelle, 1999. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon diet heart study. Circulation, 99: 779-785.
4. Gerster, H., 1998. Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? Int. J. Vitam. Nutr. Res., 68: 159-173.
5. Ogawa, A., Y. Suzuki, T. Aoyama and H. Takeuchi, 2009. Dietary alpha-linolenic acid ınhibits angiotensin-converting enzyme activity and mRNA expression levels in the aorta of spontaneously hypertensive rats. J. Oleo Sci., 58: 355-360.

6. Alessandri, C., P. Pignatelli, L. Loffredo, L. Lenti and M. del Ben *et al*., 2006. Alpha-linolenic acid-rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol., 26: 2577-2578.
7. Ren, J. and S.H. Chung, 2007. Anti-inflammatory effect of α-linolenic acid and its mode of action through the inhibition of nitric oxide production and inducible nitric oxide synthase gene expression via NF-κB and mitogen-activated protein kinase pathways. J. Agric. Food Chem., 55: 5073-5080.
8. Maden, M. and V. Aslan, 1999. [The importance of urinary enzyme activities in dogs with experimentally induced gentamicin nephrotoxicity]. Turk. J. Vet. Anim. Sci., 23: 29-42, (In Turkish).
9. Hosaka, E.M., O.F.P. Santos, A.C. Seguro and M.F.F. Vattimo, 2004. Effect of cyclooxygenase inhibitors on gentamicin-induced nephrotoxicity in rats. Braz. J. Med. Biol. Res., 37: 979-985.
10. Carrier, D., M.B. Khalil and A. Kealey, 1998. Modulation of phospholipase A_2 activity by aminoglycosides and daptomycin: A Fourier transform infrared spectroscopic study. Biochemistry, 37: 7589-7597.
11. Lee, K.E., E.Y. Kim, C.S. Kim, J.S. Choi and E.H. Bae *et al*., 2013. Macrophage-stimulating protein attenuates gentamicin-induced inflammation and apoptosis in human renal proximal tubular epithelial cells. Biochem. Biophys. Res. Commun., 434: 527-533.
12. Poljakovic, M., M.L. Svensson, C. Svanborg, K. Johansson, B. Larsson and K. Persson, 2001. *Escherichia coli*-induced inducible nitric oxide synthase and cyclooxygenase expression in the mouse bladder and kidney. Kidney Int., 59: 893-904.
13. Esposito, E. and S. Cuzzocrea, 2007. The role of nitric oxide synthases in lung inflammation. Curr. Opin. Invest. Drugs, 8: 899-909.
14. Ahn, J.M., S.J. You, Y.M. Lee, S.W. Oh and S.Y. Ahn *et al*., 2012. Hypoxia-inducible factor activation protects the kidney from gentamicin-induced acute injury. PloS One, Vol. 7. 10.1371/journal.pone.0048952
15. Ozbek, E., 2012. Induction of oxidative stress in kidney. Int. J. Nephrol. 10.1155/2012/465897
16. Zhang, W., F. Fu, R. Tie, X. Liang and F. Tian *et al*., 2013. Alpha-linolenic acid intake prevents endothelial dysfunction in high-fat diet-fed streptozotocin rats and underlying mechanisms. Vasa, 42: 421-428.
17. Ishimura, N., S.F. Bronk and G.J. Gores, 2004. Inducible nitric oxide synthase upregulates cyclooxygenase-2 in mouse cholangiocytes promoting cell growth. Am. J. Physiol.-Gastrointest. Liver Physiol., 287: G88-G95.
18. Watkins, B.A., 1991. Importance of essential fatty acids and their derivatives in poultry. J. Nutr., 121: 1475-1485.
19. Malis, C.D. and J.V. Bonventre, 1986. Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. A model for post-ischemic and toxic mitochondrial damage. J. Biol. Chem., 261: 14201-14208.
20. Rordorf, G., W.J. Koroshetz and J.V. Bonventre, 1991. Heat shock protects cultured neurons from glutamate toxicity. Neuron, 7: 1043-1051.
21. Verity, M.A., 1993. Mechanisms of phospholipase A_2 activation and neuronal injury. Ann. N. Y. Acad. Sci., 679: 110-120.
22. Bae, E.H., I.J. Kim, S.Y. Joo, E.Y. Kim and J.S. Choi *et al*., 2014. Renoprotective effects of the direct renin inhibitor aliskiren on gentamicin-induced nephrotoxicity in rats. J. Renin Angiotensin Aldosterone Syst., 15: 348-361.