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LIPID PEROXIDATION, ANTIOXIDANT PROFILE AND SOME BIOCHEMICAL PARAMETERS IN RAT APPLIED AMINOGLYCOZIDE

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ABSTRACT

Amikacin were used for the treatment of infections caused by Gram (-) bacteria, have serious nephrotoxic and ototoxic side-effects. Effects of the amikacin administrated at therapeutic doses on blood lipid peroxidation, antioxidant profile and some biochemical parameters were observed in this study. A total of 40 female Wistar albino rats were used. Amikacin (15mg/kg/day i.p) was administered to the rats in the study group and physiological saline was administered to the rats in the control group for 14 days. Within these samples, the malondialdehyde (MDA), the glutathione (GSH), the vitamin A, E, C, ceruloplasmin, albumin, globulin, total protein, alkaline phosphatase (ALP), glucose, total bilirubin and blood urea nitrogen (BUN) levels were measured. The GSH, glucose, albumin and BUN levels in the study group were statistically significantly decreased compared to the first blood collection and to the control group ($p<0.05$), and the total bilirubin level was increased. The changes in MDA, vitamin A, E, C, ceruloplasmin, ALP, creatine and globulin levels were not significant ($p>0.05$). It was observed that amikacin did not affect lipid peroxidation formation within the blood; however it changed some antioxidant levels and biochemical parameters.

KEYWORDS: Amikacin, Antioxidant, Lipid peroxidation, Biochemical parameters, Rat.

INTRODUCTION

Aminoglycoside antibiotics are widely used in veterinary and human medicine, particularly for the treatment of gram-negative bacterial infections. Aminoglycosides are proper for once

daily administration due to their concentration-related bactericidal effects and post-antibiotic effects (PAE). The daily dose is administered as a whole.[1, 2]

The therapeutic and dose-related toxicity rates of aminoglycosides are fairly high. They commonly cause nephrotoxicity, ototoxicity and neuromuscular blockage. Therefore, their clinical use is limited. The side effect nephrotoxicity is observed at a rate of 5-10% among treatments with aminoglycoside antibiotics.[3]

Factors affecting the nephrotoxicity risk of the aminoglycoside amikacin are the dose, the duration of the treatment, the genetic predisposition, age, previous aminoglycoside use and concomitant use with other nephrotoxic drugs. Generally, nephrotoxicity regresses when the drug is stopped; however the ototoxicity is irreversible.[1]

In studies conducted on the aminoglycosides, an increase in the reactive oxygen species (ROS) is found to be a factor in toxicity, which is directly related to lipid peroxidation.[2, 4] Lipid peroxidation begins with the loss of one hydrogen atom from unsaturated fatty acid chains within the membrane structure as a result of ROS formed in the organism. The membrane damage observed as a result of lipid peroxidation is irreversible. The most important peroxidation product is malondialdehyde (MDA).[5-8]

Under normal physical conditions, the organism has a complicated antioxidant defense system in order to prevent the formation of ROS, which is formed by endogenous and exogenous sources and causes oxidative damage, and as a result, injury due to these molecules. Antioxidants are classified as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants are enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The non-enzymatic antioxidants are glutathione (GSH), tocopherol, β-carotene, ascorbic acid, uric acid, cysteine, ceruloplasmin, transferrin and albumin.[9]

The biochemical blood profile of the patient should be followed-up for the diagnosis and follow-up of the patient, and the response to the therapy. Blood parameters such as glucose, urea, creatinine, cholesterol, triglyceride, albumin, globulin, total protein, lipid and bilirubin, electrolytes such as Ca, Na, K and P, and enzymes such as AST, ALT, ALP and CK are the most important parameters defining the biochemical profile. These parameters have a key role in the diagnosis, treatment and prognosis.[10]

Some of the drugs used in therapeutic doses may lead to changes in hematological and biochemical parameters. The aim of this study was to define and evaluate the changes in lipid peroxidation, antioxidant profile and some biochemical parameters in amikacin-administered rats.

MATERIALS and METHODS

Animal material: The material of this study comprised a total of 40 female Wistar- Albino rats weighing 200-250 g. The rats were kept in cages at 22±2 ºC temperature with 12 hours dark-12 hours light period and continuous fresh food and water. This study was approved by YÜHADYEK at 12.08.2010 and with approval no:07.

Designing the study groups: The rats were divided into two groups as the study (n=30) and the control (n=10) groups following the first blood collection, at the beginning of the study. The rats in the study group received amikacin (15mg/kg/day i.p.) for 14 days at the same time of the day. The rats in the control group were administered physiological saline at the same amount.

Collection of the blood samples: At the beginning and the end of the study, which took 14 days, the blood samples were collected into anticoagulant-coated vacuum tubes including gel from the left ventricles of the rats. The samples collected at the beginning of the study were evaluated as the first blood collection, and those collected at the end of the study were evaluated as the final blood collection.

Measurement of MDA[11] and GSH[12] in the whole blood and ascorbic acid[13] and ceruloplasmin[14] in the serum were made using a spectrophotometer, and measurements of vitamin A and E[15] were performed using the high performance liquid chromatography (HPLC).

Analyses of albumin, globulin, total protein, ALT, ALP, glucose, total bilirubin and BUN were performed via the auto analyzer using the Abaxis VetScan Diagnostic kit.

Statistical analysis: The results were calculated as mean±standard deviation. The statistical significance values of the data were determined by the Duncan's test.

RESULTS

The results obtained in this study have been presented in Table 1. The GSH, glucose, albumin and BUN levels in the study group were statistically significantly decreased compared to the first blood collection and to the control group ($p<0.05$), the total bilirubin was increased and glucose level in study group increases compare to control group, but the increment is not significant statistically. It was observed that the glucose level was the lowest in the study and the control groups in the final blood collection, and that this decrease was statistically significant compared to the first blood collection ($p<0.05$). No significant difference was observed between the study and the control groups with regard to MDA, vitamin A, E, C, ceruloplasmin, ALP, creatinine and globulin levels ($p>0.05$).

Table 1: First blood collection, final blood collection and parameters that belong to the study group.

Parameters	n	First blood collection S±SE	n	Final blood collection Control group S±SE	n	Final blood collection Study group S±SE
MDA (nmol/ml)	40	1.172±0.053	10	1.341±0.169	30	1.431±0.128
GSH (mg/dl)	40	0.742±0.024 b	10	0.665±0.0407 b	30	0.557±0.023 a
Vitamin-A (mg/dl)	40	0.637±0.045	10	0.631±0.035	30	0.695±0.205
Vitamin-E (mg/dl)	40	2.754±0.267	10	2.766±0.312	30	2.743±0.237
Vitamin-C (mg/dl)	40	1.536±0.102	10	1.441±0.132	30	1.410±0.079
Ceruloplasmin(%mg)	40	58.800±2.372	10	49.670±2.580	30	52.765±3.326
Albumin (g/L)	40	62.000±7.632 b	10	59.000±8.165 b	30	55.800±1.172 a
ALP (U/L)	40	255.786±9.184	10	257.857±18.593	30	280.067±15.941
Creatinine (umol/L)	40	41.571±2.245	10	41.142±2.324	30	44.200±1.200
Total Bilirubin (umol/L)	40	3.214±0.114 a	10	3.429±0.202 ab	30	3.867±0.215 b
BUN (mmol/L)	40	8.686±0.351 b	10	7.600±0.996 b	30	6.000±0.322 a
Glucose (mmol/L)	40	9.764±0.289 b	10	8.143±0.121 a	30	8.607±0.279 a
Globulin (g/L)	40	20.429±0.844	10	19.286±1.190	30	20.000±1.215

$^{a.b}p < 0.05$

DISCUSSION

It has been reported that the aminoglycoside class of antibiotics have the ability of forming reactive oxygen radicals (ROS), and that ROS affects the appearance of the side effects formed by these antibiotics.[2]

Aminoglycoside-related nephrotoxicity is characterized by direct tubular damage and an increase in the glomerular filtration rate. The reaction between the cationic aminoglycosides and anionic membrane phospholipids has been accepted as the first cytotoxic step.[4]

It has been reported that aminoglycoside-induced nephrotoxicity is related to lipid peroxidation, and that the aminoglycoside class of antibiotics may cause damage in the renal tissue in long-term high-dose use.[16]

As a substrate for reactive oxygen radicals, MDA is the final product of the lipid peroxidation formed as a result of catabolization of the unsaturated fatty acids.[8]

Cells and tissues have a system that inhibits the radical products and reactions. Substances that rapidly react with the radicals and prevent the progression of auto-oxidation/peroxidation have been referred to as antioxidants.[17]

Glutathione is an important antioxidant compound of the cells and the glutathione present within the cell is rapidly consumed when stress is increased for any reason.[18] Ceruloplasmin, which is mainly synthesized in the liver, is an acute phase protein that moderately responds to inflammation and tissue damage.[19] Antioxidant vitamins such as vitamin E, C or carotenoids work together effectively when they are taken with foods, and eliminate the harmful effects of reactive oxygen species that cause diseases or injuries.[8]

Although many studies have been conducted with gentamicin, there are very few studies on the other aminoglycoside antibiotics.

In the study of Ertekin et al.[20] on dogs in which gentamicin (15 mg/kg i.m) was administered for 10 days, significant increases were observed in the levels of serum MDA, ceruloplasmin and β- carotene compared to pre-treatment, whereas significant decreases were observed in the levels of GSH and vit E. Changes in the levels of retinol and vit C were not significant.
It has been reported that gentamicin (100 mg/kg/day i.p)-administered rats have presented with acute nephrotoxicosis, and increased plasma MDA and nitric oxide (NO) levels.[21]

In another study, the serum MDA levels were non-significantly increased in the gentamicin-administered group (20 mg/kg im) compared to the control group, and the total serum antioxidant activity was significantly reduced.[22]

Karahan et al.[23] observed significant increases in the plasma MDA and blood GSH levels of rats after a single dose administration of cisplatin (7 mg/kg).

It was reported that administration of different aminoglycoside antibiotics (amikacin, gentamicin, kanamycin and streptomycin) had no effect on the SOD and GPX activities within the renal tissue and the MDA level, but decreased the GSH level.[16]

It was reported in studies with amikacin that the renal MDA level was increased[24, 25] and the GSH level was decreased[24] compared to the control group.

Karahan et al.[26] reported that a significant increase was observed in the MDA levels within the renal tissue and no change was observed in the GSH levels, in the rats receiving gentamicin (100 mg/kg) for 6 days.

In the presented study, the GSH level was significantly decreased ($p<0.05$) and the MDA, vitamin (A, E and C) and ceruloplasmin levels were non-significantly changed ($p>0.05$) in the study group compared to the first blood collection and the control group. The decrease in the GSH level may be related to the oxidative stress observed as a result of drug administration. Because GSH primarily defended the cell probably against the toxic effects of hydroxyl radicals and singlet oxygen, there was no need for the protective effects of other antioxidants like vitamin C and E with the therapeutic dose of the drug.

It was thought that the changes in the parameters in the control group depend on the stress occurred during injection. It is well known that stress increases adrenaline secretion and the adrenalin secretion caused by the changes on GSH, MDA and glucose.[27, 28]

Nephrotoxicity is a side effect that may develop for all aminoglycoside antibiotics and may be observed at a rate of 5-10% in aminoglycoside use.[3]

Aminoglycosides lead to thromboxane B_2 - controlled renal vasoconstriction and cause direct cellular toxicity in the proximal tubules where drugs are absorbed and kept within the lysosome. Therefore, tubular necrosis, tubular atrophy, intra-tubular myeloid substances and interstitial nephritis may develop.[29-33]

The nephrotoxic effect is commonly observed with gentamicin, followed by tobramycin, amikacin and nethylmycine, respectively.[33]

The creatinine and BUN tests are used together in order to evaluate the renal function, help to diagnose the renal disease and to follow-up the acute or chronic renal failure.[34, 35]

There are studies supporting and not supporting the hypothesis that aminoglycoside antibiotics lead to permanent or transient changes in the blood parameters, depending on the renal functions.[36]

Liver is the primary organ that synthesizes the plasma proteins albumin and globulin. Serum albumin and globulin tests are used to investigate hepatic functions. An absolute increase in serum albumin is not normally observed. This may result from decreased albumin level, advanced hepatic disorders, amino acid absorption failure or insufficient dietary intake of proteins. Increase in serum globulin level is observed in acute and chronic hepatic disorders, chronic infections, acute diffuse glomerulonephritis, sarcoidosis, carcinomas and autoimmune diseases.[10, 37]

Bilirubin is formed as the result of hemoglobin degradation in the liver, spleen and bone marrow. The bilirubin level is increased in all diseases causing hepatocellular damage and/or cholestasis in the liver, and in the obstruction of extra-hepatic biliary ways.[38]

ALP is synthesized in the liver, bone, kidneys and the placenta. Changes in serum and plasma ALP activity may depend on hepatic damage, gall bladder damage, use of steroid hormones or some types of drugs, as well as toxicity with heavy metals such as lead or mercury, or malnutrition.[10, 38]

Some aminoglycoside type antibiotics have been reported to be ineffective on the levels of serum creatinine, potassium, sodium, total protein, glucose, uric acid and total bilirubin.[16] Increased activities of serum GOT and GPT, and increased levels of uric acid, total bilirubin and creatinine have been reported in amikacin-induced nephrotoxicosis and hepatotoxicity, which have been confirmed histologically.[25] Slightly increased serum BUN levels and unaffected serum albumin levels have been reported in amikacin-induced nephrotoxicosis.[24] Sinwast et al.[39] reported a significant increase in BUN levels and insignificant changes in albumin and creatinine levels in gentamicin-induced ototoxicity.

In nephrotoxicosis induced by different doses of gentamicin, significantly increased urea and creatinine levels have been reported in dogs[20, 22] and rats.[26, 40]

Aminoglycosides have been reported to be more effective than aminocyclitol since kidney function-related parameters have changed following the administration of aminoglycosides and aminocyclitol at therapeutic doses in goats. It was reported that the plasma creatinine

(aminoglycoside) and urea (aminoglycoside and aminocyclitol) levels were increased and glucose level was decreased, whereas the total protein level and alkaline phosphatase activity demonstrated various changes.[36]

In the presented study, the albumin and BUN levels in the study group were statistically significantly decreased compared to the first blood collection and to the control group ($p<0.05$), and the total bilirubin level was increased. The decrease in the albumin level may be related to damage to the liver or its use as an antioxidant.

The glucose level was found to be lowest among the study and control groups, which was statistically significant compared to the first blood collection ($p<0.05$). Oxidative stress, which was formed secondary to the applications, may have led to hypoglycemia. The ALP, creatinine and globulin levels were not significantly different between groups ($p>0.05$).[28]
In conclusion, amikacin used at therapeutic dose did not affect lipid peroxidation formation; however, it changed some antioxidant levels and biochemical parameters.

CONCLUSIONS

In this study, the effects of amikacin administrated at therapeutic doses in rats on blood lipid peroxidation, antioxidant profile and some biochemical parameters were examined. It was observed that amikacin did not affect lipid peroxidation formation within the blood, However, some antioxidant levels and biochemical parameters changed.

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