

IN VIVO EVALUATION OF AMINOGLYCOSIDE INDUCED NEPHROTOXICITY AND HEPATOTOXICITY IN ALBINO RATS

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Summary

The objective of the present study was to evaluate both qualitatively and quantitatively the toxic effect of amikacin, on rat's kidney and liver via measuring the in vivo concentration of the antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase, creatinine along with malonaldehyde (MDA), total bilirubin level and uric acid levels in albino rats. Oxidative stress and free radical are causative factors for aminoglycoside induced renal and hepatic injury. Observed results showed that the activities of the antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase were significantly lowered along with increase in the MDA levels after the treatment with amikacin as compared to control group (normal saline). Amikacin has also found to increase other biochemical parameters such as SGOT, SGPT, Uric Acid, Total Bilirubin and Creatinine in the treated group as compared to control group. The results were further supported by the histological reports. These findings suggested that the treatment with aminoglycoside such as amikacin leads to a significant free radical generation which leads to oxidative stress, resulting into a significant hepatic and renal tissue injury.

Keywords: Amikacin, SGOT, SGPT, Total Bilirubin, Creatinine, Reactive Oxygen Species

Introduction

Aminoglycoside antibiotics have long been used in antibacterial therapy. It has been reported that amikacin induce free radical generation which implicates a variety of pathological processes¹. Amikacin generate free oxygen radicals, leading to tissue injury such as nephrotoxicity and ototoxicity². It has been reported that renal damage can in turn leads to liver injury due to aminoglycosides³⁻⁴. Aminoglycosides (amikacin) are potentially toxic at levels only slightly above therapeutic range^{1,5}. The association of aminoglycosides with negatively charged phospholipids and their accumulation in the lysosomes of tubular cells leads to phospholipidosis by inhibition of lysosomal phospholipases⁶, which may trigger necrosis⁷. It has been reported that at cellular level, aminoglycosides interferes with protein synthesis, especially by inhibition of translocation⁸.

The superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) can stimulate free-radical reactions. Superoxide dismutases (SOD) remove O_2^- by greatly accelerating its conversion to H_2O_2 . Catalases in peroxisomes convert H_2O_2 into water and O_2 and help to dispose of H_2O_2 generated by the action of the oxidase enzymes that are located in these organelles. Other important H_2O_2 -removing enzymes in human cells are the glutathione peroxidases. When produced in excess, ROS can cause tissue injury. However, tissue injury can itself cause ROS generation. The purpose of present study was to evaluate effect of amikacin on renal and hepatic tissues, comparing the same with that of control groups treated with 0.9 % w/v normal saline solution.

Materials and methods

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

Twelve 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 2 groups of six rats each as given below:

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

The respective drugs were administered intramuscularly for 28 days. At the end of treatment, 1 ml blood sample were drawn in sodium citratised 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 °C before performing the enzyme assay.

Superoxide dismutase (SOD) assay

SOD activity was determined by the Method of Fradovich and Misra⁹. 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°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 assay

Catalase activity was measured by the method of Luck¹⁰. 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°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°C.

Measurement of lipid peroxidation

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldehyde (MDA) formed, essentially according to Ohkawa et al.¹¹. MDA is the most abundant individual aldehyde resulting from lipid peroxidation. It was determined by thiobarbituric 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°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, Gujurat India).

Glutathione reductase assay

Glutathione reductase activity was measured by the method of Carlberg and Mannervik¹². 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 6.22×10^3 .

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 °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. Tissue sections were 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.

Statistical analysis

All values are expressed in Mean + SEM. Unpaired t-test with Welch correction was used to determine statistical difference between control and experimental groups. P<0.05 was considered statistically significant.

Results

In the present study toxic effect of Amikacin on rat liver and kidney was observed and shown in table 1.

Table 1: Effect of amikacin on various biological parameters

Parameters	Control	Amikacin
SOD	810 + 2.033	250 + 2.933 ^a (- 69.13%)
Catalase	187 + 1.932	111 + 2.324 ^a (- 40.64%)
MDA	656.43 + 2.173	875 + 2.781C ^a (+ 33.38%)
Glutathione Reductase	1.52 + 0.0152	0.73 + 0.0173 ^a (- 51.97 %)
Creatinine	0.95 + 0.1317	1.4 + 0.1668 ^c (+ 47.36 %)
Total Bilirubin	0.512 + 0.0015	0.634 + 0.0015 ^a (+23.82%)
Uric Acid	2.7 + 0.1673	3.7 + 0.1653 ^b (+37.03%)
SGPT	45 + 1.880	139 + 2.280 ^a (+208.88%)
SGOT	43 + 1.317	97 + 2.595 ^a (+125.58%)
Values are expressed in Mean (+) SEM for 6 rats in each group where ^a p<0.0001, ^b p<0.001, ^c p< 0.05 were regarded as significant as compared to control. Values in the parenthesis indicates the percent change in biochemical parameters as compared to control.		

The results of the present study showed a significant decrease in Superoxide Dismutase (69.13%), Catalase (40.64%), Glutathione Reductase (51.97 %) activity along with increase in the level of various biological parameters such as MDA (33.38 %), Creatinine (47.36%), Total Bilirubin (23.82 %), Uric Acid (37.03 %), SGPT (208.88 %) and SGOT (125.58%) in amikacin treated group as compared to control group.

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.

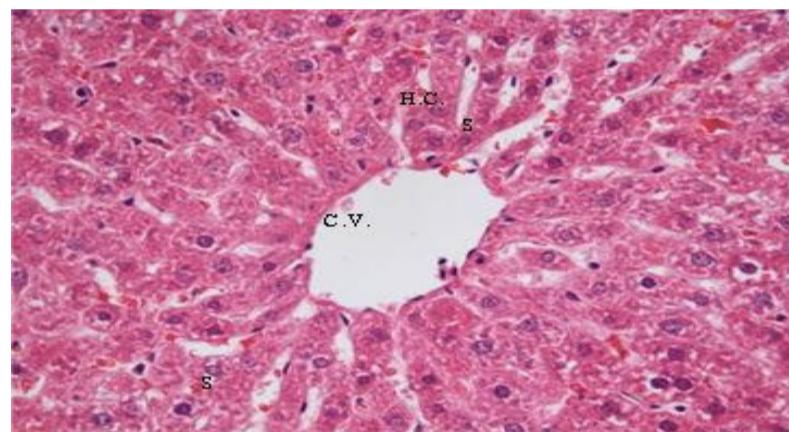


Figure 1: Liver tissue of control showing normal structure, central vein (C.V.), normal arrangement of hepatic cords (H.C.), normal blood sinusoids (S) and hepatocytes. HE, X 400.

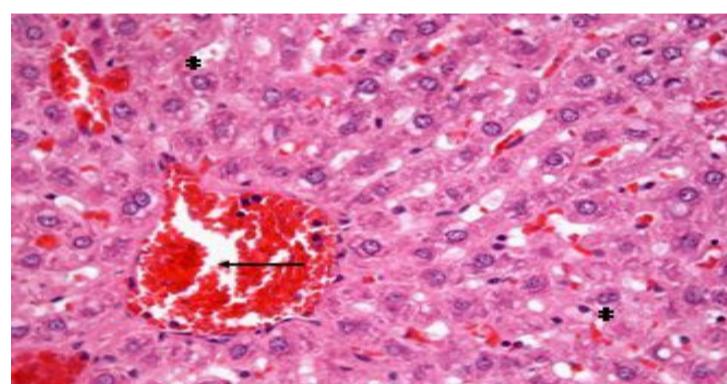


Figure 2: Liver tissue of Amikacin treated rats showing haemorrhage in the central vein (-) with dilated blood sinusoids and necrosis. HE, X 400.

Discussion

Free radical and their derivative 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^{13,14}. Aminoglycosides enhance excessive production of hydrogen peroxide by the renal cortex which inhibit the synthesis of phospholipases A2 and glutathione in rats¹⁵. 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.

Various other studies have been also reported that amikacin and tobramycin alter antioxidant defence mechanism¹⁶. Aminoglycosides are rich in primary amines and possess cytoprotective properties but would not be expected to protect extracellular sulphhydryl group against free radical-mediated oxidation.

The present finding demonstrate that single treatment with amikacin, which is an antibiotic drug of aminoglycosides category significantly lowered the antioxidant enzymes activities (SOD, Catalase and 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. These biological parameters indicate that administration of single antibiotic causes the renal toxicity. Similar finding was reported with other aminoglycosides such as streptomycin and gentamycin in kidney and heart^{17,18}.

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. Dilated glomeruli show mild hypercellularity and congestion in most sections. Infiltration of polymorphonuclear and mononuclear leukocytes, lymphocytes, plasma cells and macrophages. 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.

Conclusion

The study concluded that in spite of its good efficacy as an antibiotic, the amikacin which is very popular drug from aminoglycoside category has been found to cause a severe damage to the nephrotic and hepatic tissue, at a dose just above the therapeutic range.

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