Streptomycin induced nephrotoxicity: Generation of free radicals and antioxidant effect of vitamin C

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Abstract

Introduction: Streptomycin is used in many conditions like tuberculosis, plague, tularemia and peritonitis. Kidney damage is one of the feared complications of streptomycin. Free radicals are implicated in many pathology including renal diseases. In vitro studies of aminoglycosides have demonstrated potential to generate free radicals.

Aims: The study was aimed at exploring oxidative stress induced during kidney damage by streptomycin and benefit of antioxidant vitamin C.

Materials and Methods: Total 28 Wistar male albino rats were used in the study were divided into 4 groups. In two groups saline i.m. 0.3 ml/day or vitamin C i.p.200 mcg/kg/day were given. In another two group Streptomycin 100 mg/kg/day i.m. was given either alone or along with vitamin C. Oxidative stress and generation of free radicals were measured by Serum total antioxidant status (TAS), glutathione peroxidase (GPx), glutathione reductase (GR), while blood urea and serum creatinine were measured for estimation of renal damage. Rat kidneys were examined for hisptopathology.

Statistical analysis: The data was subjected to one way ANOVA with post hoc test of significance and the values of $P < 0.05$ were considered statistically significant.

Results: Administration of streptomycin led to significant fall in the values of TAS, GPx and GR while there was significant rise in the value of blood urea and s. creatinine. TAS level was elevated significantly in vitamin C treated group. When vitamin C was given along with streptomycin there was significant prevention of fall in GPx, GR and TAS while rise in blood urea and s. creatinine was prevented.

Conclusions: Free radicals are generated during treatment with streptomycin leading to nephrotoxicity which was significantly prevented by coadministration of vitamin C.

Keywords: Streptomycin, free radicals, Glutathione peroxidase, Glutathione reductase, Total antioxidant status, Nephrotoxicity.

Introduction

Streptomycin is one of the important aminoglycoside for treatment of tuberculosis, plague, peritonitis as well as other gram negative infections including UTI. Possible kidney damage is one of the limiting factors associated with the use of aminoglycoside like gentamicin and streptomycin. The duration and dose of therapy is always delineated due to the kidney damage. All aminoglycosides produce renal damage of varying degree due to their accumulation in cells of proximal convoluted tubules.[1]

Generation of free radicals and injury due to free radicals is implicated in a number of pathogenic processes including nephropathy.[2-4] So it was conceived to examine possibility of generation of free radicals and their involvement in inflicting kidney damage by widely used aminoglycoside streptomycin in experimental animal model. Further it was also planned to explore the efficacy of an antioxidant agent[5,6] vitamin C to prevent and/or to reverse the structural damage in kidneys resulting due to streptomycin.

Materials and Methods

Around 18 weeks old adult, male albino wistar strain rats were used with an average weight of around 175 gms. Food and water was given as per their daily requirement. After approval from the animal ethics committee the experiment was done. There were 4 groups, with 7 rats in each. The drug administered was as follows:

Group I Control group, was given saline 0.3 ml i.p. per day. Group II was given Vitamin C 0.2mg/kg/day i.p. Group III was given streptomycin 100 mg/kg/day i.m. While group IV received streptomycin and vitamin C with 100mg/kg/day i.m. and 0.2 mg/kg/day i.p. respectively. Total duration of treatment was 30 days with single daily dose of each drug.

Collected samples

Sodium thiopentone 50 mg/kg i.p. was given for euthanasia, and blood was collected from heart with puncture and stored in heparinized bulbs and plain bulbs. Kidney sample was kept in 10% formalin.

Biochemical parameters

Blood urea and s. creatinine were measured to estimate renal damage. To measure oxidative stress Glutathione peroxidase (GPx),[7] Glutathione reductase (GR)[8] and Total antioxidant status (TAS)[9] were analyzed. These items were bought from Randox laboratory, U.K. Spectrophotometry was used for analysis.

Statistical analysis

One way ANOVA with post hoc analysis was done. $P<0.05$ was considered statistically significant.

Results

Results are depicted in table – 1.

Oxidative stress

There was an increase of GPx by 9% in vitamin C group, while GR and TAS were increased by 4% and 28% respectively as compared to control. In streptomycin treated group all values were significantly decreased as can be seen from tables. Co-administration of vitamin C significantly prevented oxidative stress and reduced formation of free radicals.

Renal damage

There was significant rise in the level of blood uear and s.creatinine in streptomycin treated group. When vitamin C was co-administered changes were less evident.

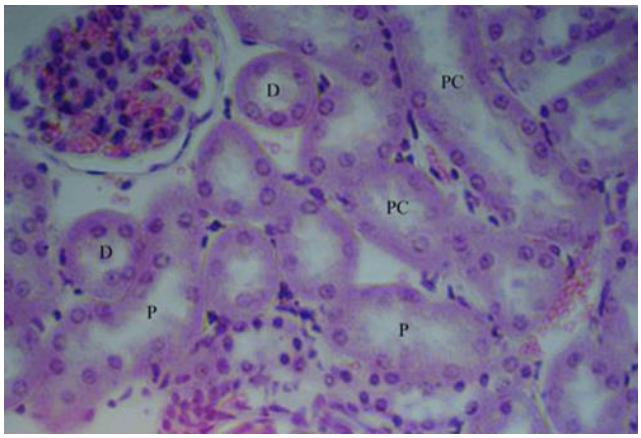

Fig. 1: Picture of saline treated normal rat kidney nephron with cortex and tubules (H&E 400X)

In the renal structure Proximal tubules (P) and distal tubules (D) are visible. Distal tubules have smaller diameter as compared to proximal tubules. There is presence of brush border in proximal tubules while distal tubules are devoid of brush border with clearer luminar surface. A small part of glomerulus is also seen in same picture.

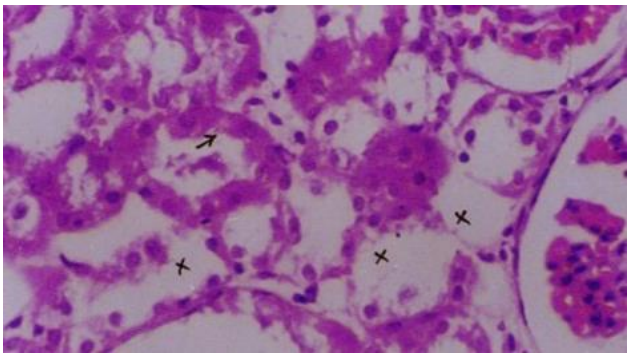

Fig. 2: Picture of rat kidney treated with streptomycin (H&E 400X)

The proximal tubular structure shows remarkable damage with derange epithelia. Cellular necrosis is found in many proximal tubules. Prominent damage to renal brush border is seen. Distal tubule are showing less disruption (arrow).

Table 1: Effect of streptomycin and vitamin C induced changes in blood urea, serum creatinine, glutathione peroxidase, glutathione reductase and total antioxidant status

Group	Drug treatment	B. urea mg%	S. creatinine mg%	Glutathione Peroxidase U/l	Glutathione Reductase U/l	Total Antioxidant Status mmol/l
I	Saline	39±2.04	0.57±0.14	1020±41	73±12.15	1.28±0.04
II	Vitamin C	40±2.73	0.61±0.15	1099±71	80±4.83	1.64±0.05 ***
III	Streptomycin	46±1.6 *	1.28±0.11 *	767±26 ***aa	48±2.3 *aa	1.15±0.02 ***aaa
IV	Streptomycin + Vitamin C	40±1.1*b	0.59±0.14 bb	875±38 *a	65±2.8 b	1.26±0.03aaabbb
ANOVA F ; d P value		3.019 ; 3,28 0.0494	6.744 ;3,28 0.0019	9.85 ;3,28 0.0002	4.27 ;3,28 0.0149	70.58 ;3,28 0.0001

Values are mean ± SEM; *P<0.05, **P<0.01 and ***P<0.001 when compared to group I.
a P<0.05, aa P<0.01 and aaa P<0.001 when compared to group II.
b P <0.05,bb P<0.01 and bbb P<0.001 when compared to group III

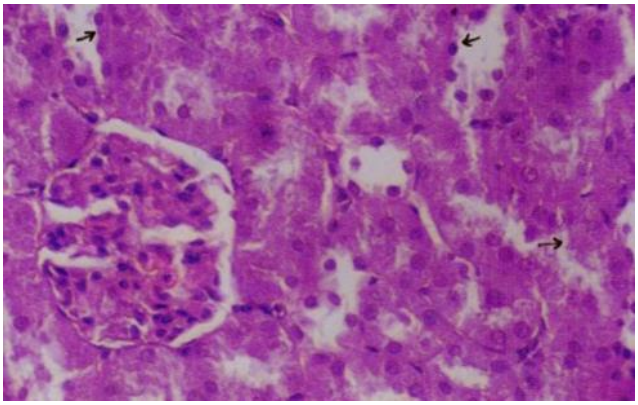

Fig. 3: Picture of streptomycin along with vitamin C treated rat kidney (H&E 400X)

Larger proximal tubules display less damage (arrow), along with vital epithelium. In some PCT only damage to brush border is seen while integrity of brush border in other is well maintained. Preservation of normal cellular structure is evident.

Discussion

Streptomycin is one of the drugs in treatment of tuberculosis, plague and other diseases due to gram negative organisms. In renal glands streptomycin gets reabsorbed and get concentrated in cells of proximal tubules. High value of trough is responsible for the renal damage. Mechanism of renal damage is less explored, but there is skepticism about the role of oxidative stress and free radical generation. Coadministraion of vitamin C can be of some benefit in preventing renal damage due to its scavenging action exerted on free radicals.[10-12]

Histopathological examination revealed kidney damage produced by administration of streptomycin in present study. Renal structure was disrupted especially in proximal convoluted tubules. Marked degenerative changes were observed. In some tubular cells piknotic nuclei were found suggesting patchy necrosis. Recently involvement of free radicals is under scanner in many diseases owing to their ability of cellular disruption.[13-15]

Free radicals are highly reactive due to unstable electron in their outer orbital ring, and can pause a potential threat to integrity of any cellular structure.[16] GPx and GR are powerful antioxidants and prevent formation of free radicals and thereby also prevent lipid peroxidation as well as cell damage. TAS is the value of individual's ability to fight against oxidative stress produced by free radicals.

Treatment with streptomycin for 30 days there was significant reduction in the value of all the parameters of oxidative stress namely GPx, GR and TAS. Leonard et al[17] also observed renal damage characterized by acute tubular necrosis after using tobramycin 200mg/kg/day. Sens et al[18] studied effect of increasing concentration of streptomycin on the human proximal tubular cell culture. They monitored for cell death, light and electron microscopic changes under both resting and actively dividing culture conditions. Damage to renal brush border and microvilli was evident as this one is active site for drug transport. Kavutcu et al also found similar reduction in GPx level in guinea pig while studying effect of gentamicin.[19]

Reduction in antioxidant level is totally reflected in the value of TAS. Once established the involvement of oxidative stress in renal injury due to streptomycin it was necessary to examine the role of antioxidant vitamin C which is natural antioxidant and free radical scavenger.[20-22] Lemon, orange, strawberries like fruits are rich in vitamin C having antioxidant ability. Free radicals like peroxyl and hydroxyl radicals are scavenged by vitamin C.[23]

In present study it was found that there was significant increase in GPx and GR level. Hong et al observed protective role of vitamin C at 0.18 mg/kg/day in nephrotoxicant injury.[24] Bradberry and Vale found similar protective role of vitamin C at 0.5 mg/kg/day in chromium induced renal damage. [25]

In removal of free radical there is competition between vitamin C and GPx or GR enzymes. As a result use of vitamin C reduces fall in the level of GPx as well as GR level and as a result overall increase in TAS value. In present study rise in TAS level in vitamin C treated group supports antioxidant ability of vitamin C. In vitro studies have shown ability to generate free radicals and induce oxidative stress with aminoglycoside antibiotics.[26] In renal cortical mitochondria, formation of free radical like super oxide as well as hydrogen peroxide have been associated with aminoglycosides.[27-29] A notorious hydroxyl radical is formed due to interaction between super oxide anion and hydrogen peroxide which can pause a threat to mitochondrial DNA as well as matrix. Formation of free radicals can also damage other cellular organelle and cell membrane. All these can lead to leakage of protein from inside out.12

Zima et al compared the action of free radical with second messanger system and formation of various interleukins which can ultimately enhance proteolytic activity, damage collage and extracellular matrix in glomerular structure.[30] In present study rise in level of blood urea and s. creatinine was suggestive of renal damage due to streptomycin.

Antioxidant vitamin C has shown predictive benefit in streptomycin induced nephrotoxicity in present study however more extensive studies are desirable to arrive to a definite conclusion.

Source of Funding

None.

Conflict of Interest

None.

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