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Assessment of antioxidant effect of vitamin c in on free radicles generated in streptomycin induced nephrotoxicity

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Abstract
Oxidative stress has been recognized as an important contributory factor in a number of pathogenic processes including those affecting kidney leading to the possibility of utilizing the antioxidants for the prevention of nephrotoxicity. Accordingly this study was planned to investigate the possible generation of oxidative stress by Streptomycin leading to renal damage in experimental rat model. Also it was aimed at localization of the site of damage produced by Streptomycin with histopathological study of rat kidney. Antioxidants are substances capable to repair or prevent the damage produced by oxidative stress. Vitamin C is a naturally occurring powerful antioxidant. It has shown beneficial effect in many conditions where oxidative stress is generated. Hence based on above findings the present study was planned for Assessment of Antioxidant Effect of Vitamin C in on Free Radicles Generated in Streptomycin Induced Nephrotoxicity.
The present study was planned in Department of Pharmacology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India. The study was planned from duration of January 2018 to Oct 2018. Total 20 Adult, male albino wistar strain rats were enrolled in the present study. The rats were divided in four study groups. Group A Control group, was given saline 0.3 ml i.p. per day. Group B was given Vitamin C 0.2mg/kg/day i.p. Group V was given streptomycin 100 mg/kg/day i.m. While group D 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.
The data generated from the present study concludes that Antioxidant vitamin C has shown predictive benefit in streptomycin induced nephrotoxicity in recent study however more extensive studies are desirable. 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, nephron toxicity, etc

1. Introduction
Aminoglycosides preferentially affect the proximal tubular cells. These agents are freely filtered by the glomeruli and quickly taken up by the proximal tubular epithelial cells, where they are incorporated into lysosomes after first interacting with phospholipids on the brush border membranes. They exert their main toxic effect within the tubular cell by altering phospholipid metabolism. In addition to their direct effect on cells, aminoglycosides cause renal vasoconstriction.
The 2 critical factors in the development of acute kidney injury (AKI) secondary to aminoglycoside nephrotoxicity are dosing and duration of therapy. Approximately 5% of the administered dose accumulates within epithelial cells after glomerular filtration [1]. Aminoglycoside uptake by the tubules is a saturable phenomenon, so uptake is limited after a single dose. Thus, a single daily large dose is preferable to 3 doses per day. One dose per day presumably causes less accumulation in the tubular cells once the saturation point is reached [2, 3].
Extending the dose interval to > 24 hours in patients with renal impairment has been found effective, with irreversible nephrotoxicity reported in approximately 1% of the patients studied [4].
The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs [5]. Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs (Finn and Porter, 2003; Galley, 2000). Approximately 20% of nephrotoxocity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity [6].
Nephrotoxicity can be diagnosed through a simple blood test. Evaluation of nephrotoxicity through blood tests includes the measurements of blood urea nitrogen (BUN), concentration of serum creatinine, glomerular filtration rate and creatinine clearance. However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged [7].
Streptomycin is an antibiotic used to treat a number of bacterial infections. This includes tuberculosis, Myco bacterium avium complex, endocarditis, brucellosis, Bur kholderia infection, plague, tularemia, and rat bite fever. For active tuberculosis it is often given together with isoniazid,

rifampicin, and pyrazinamide. It is given by injection into a vein or muscle.

Common side effects include feeling like the world is spinning, vomiting, numbness of the face, fever, and rash. Use during pregnancy may result in permanent deafness in the developing baby. Use appears to be safe while breastfeeding. It is not recommended in people with myasthenia gravis or other neuromuscular disorders. Streptomycin is an aminoglycoside. It works by blocking the ability of 30S ribosomal subunits to make proteins, which results in bacterial death [8].

The most concerning side effects, as with other aminoglycosides, are kidney toxicity and ear toxicity. Transient or permanent deafness may result. The vestibular portion of cranial nerve VIII (the vestibulocochlear nerve) can be affected, resulting in tinnitus, vertigo, ataxia, kidney toxicity, and can potentially interfere with diagnosis of kidney malfunction [9].

Common side effects include vertigo, vomiting, numbness of the face, fever, and rash. Fever and rashes may result from persistent use. Use is not recommended during pregnancy. Congenital deafness has been reported in children whose mothers received streptomycin during pregnancy. Use appears to be okay while breastfeeding. It is not recommended in people with myasthenia gravis.

Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit [10]. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the mRNA strand. This results in an unstable ribosomal-mRNA complex, leading to a frameshift mutation and defective protein synthesis; leading to cell death [11]. Humans have ribosomes which are structurally different from those in bacteria, so the drug does not have this effect in human cells. At low concentrations, however, streptomycin only inhibits growth of the bacteria by inducing prokaryotic ribosomes to misread mRNA [12]. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria [13], and is therefore a useful broad-spectrum antibiotic.

Streptomycin was first isolated on October 19, 1943, by Albert Schatz, a PhD student in the laboratory of Selman Abraham Waksman at Rutgers University in a research project funded by Merck and Co. Waksman and his laboratory staff discovered several antibiotics, including actinomycin, clavacin, streptothricin, streptomycin, grisein, neomycin, fradicin, candicidin, and candidin. Of these, streptomycin and neomycin found extensive application in the treatment of numerous infectious diseases. Streptomycin was the first antibiotic cure for tuberculosis (TB). In 1952 Waksman was the recipient of the Nobel Prize in Physiology or Medicine in recognition "for his discovery of streptomycin, the first antibiotic active against tuberculosis". Waksman was later accused of playing down the role of Schatz who did the work under his supervision, claiming that Elizabeth Bugie had a more important role in its development [14].

The Rutgers team reported streptomycin in the medical literature in January 1944 [15]. Within months they began working with William Feldman and H. Corwin Hinshaw of the Mayo Clinic with hopes of starting a human clinical trial of streptomycin in tuberculosis. The difficulty at first was even producing enough streptomycin to do a trial, because the research laboratory methods of creating small batches had not yet been translated to commercial large-batch production. They managed to do an animal study in a few guinea pigs with just 10 grams of the scarce drug, demonstrating survival. This was just enough evidence to get Merck & Co. to divert some resources from the young penicillin production program to start work toward streptomycin production [16].

At the end of World War II, the United States Army experimented with streptomycin to treat life-threatening infections at a military hospital in Battle Creek, Michigan. The first person who was treated with streptomycin did not survive; the second person survived but became blind as a side effect of the treatment. In March 1946, the third person-Robert J. Dole, later Majority Leader of the United States Senate and Presidential nominee-experienced a rapid and robust recovery [17].

The first randomized trial of streptomycin against pulmonary tuberculosis was carried out in 1946 through 1948 by the MRC Tuberculosis Research Unit under the chairmanship of Geoffrey Marshall (1887-1982). The trial was neither double-blind nor placebo-controlled. It is widely accepted to have been the first randomised curative trial. Results showed efficacy against TB, albeit with minor toxicity and acquired bacterial resistance to the drug [18].

Oxidative stress has been recognized as an important contributory factor in a number of pathogenic processes including those affecting kidney leading to the possibility of utilizing the antioxidants for the prevention of nephrotoxicity. Accordingly this study was planned to investigate the possible generation of oxidative stress by Streptomycin leading to renal damage in experimental rat model. Also it was aimed at localization of the site of damage produced by Streptomycin with histopathological study of rat kidney. Antioxidants are substances capable to repair or prevent the damage produced by oxidative stress. Vitamin C is a naturally occurring powerful antioxidant. It has shown beneficial effect in many conditions where oxidative stress is generated [19-20].

Hence based on above findings the present study was planned for Assessment of Antioxidant Effect of Vitamin C in on Free Radicles Generated in Streptomycin Induced Nephrotoxicity.

Methodology

The present study was planned in Department of Pharmacology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India. The study was planned from duration of January 2018 to Oct 2018.

Total 20 Adult, male albino wistar strain rats were enrolled in the present study. The rats were divided in four study groups. Group A Control group, was given saline 0.3 ml i.p. per day. Group B was given Vitamin C 0.2mg/kg/day i.p. Group V was given streptomycin 100 mg/kg/day i.m. While group D 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.

At the end of treatment, the rats were sacrificed after giving sodium thiopentone 50 mg/kg i.p. and blood samples were

collected directly from the heart. The blood samples were taken in plain as well as heparinized bulbs for biochemical estimation. The kidneys were dissected out and preserved in 10 % formalin for the histopathological study.

Results and Discussion

Aminoglycoside (AG) nephrotoxicity is a well-known occurrence. However, 50 years after the discovery of the first AG (streptomycin), nephrotoxicity is still very difficult to predict and avoid [21].

To date, no model has completely described the pharmacodynamics behaviour of AG nephrotoxicity, even though the mechanism of this toxicity has been widely studied [21]. AGs are retained in the epithelial cells lining the proximal tubule after glomerular filtration. AGs become attached to the brush-border membrane in their cationic form. The initial points of attachment are probably the acidic phospholipids, especially phosphatidylserine. In this way, aminoglycosides accumulate and cause leakage of intracellular ions (K+, Mg2+, Ca2+), proteins (beta-2-microglobulin, alpha-2-macroglobulin, lysozyme), and enzymes (alanylaminopeptidase, N-acetylglucosaminidase). Thus, the resulting decline in glomerular filtration has a multifactorial origin and involves a combination of tubular and nontubular mechanisms. The most important factor seems to be a tubuloglomerular feedback [23]. The kidneys have a strong capacity to compensate for tubular injuries. The importance of regeneration for protection against renal injury is clearly demonstrated by the survival of laboratory rats exposed to repeated administrations of high daily doses of AG (40 mg of gentamicin per kg of body weight per day for at least 42 days). After an initial episode of acute tubular necrosis that occurs within 8 to 10 days and that is associated with marked azotemia, the renal function returns almost to normal, as if the kidney had become refractive [24]. Regenerating cells are less differentiated and apparently less susceptible to AG (the level of accumulation of AG is actually reduced in the cortices of animals treated for long periods).

In the recent years increasing interest is seen in the role of free radicals and oxidative damage in a variety of human diseases [25-27]. Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules which are generally very reactive [28]. Free radicals have the potential to oxidize biomolecules including proteins, lipids and DNA. The enzyme GPx is selenium dependant enzyme and its main function is removal of H2O2 and it prevents formation of highly reactive hydroxyl (OH-) radical. GR is also an important enzyme with main function of restoration of cellular glutathione level by reducing oxidized disulfide glutathione GSSG. Total antioxidant status (TAS) study gives a guideline for individual's ability to stand against oxidative stress.

The aminoglycosides are capable of generating free radicals *ein vitro*. Aminoglycoside antibiotics have been shown to enhance the generation of super oxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical. The mitochondrial DNA (mtDNA) is susceptible to oxidative damage by the everincreasing levels of ROS and free radicals in the mitochondrial matrix. Spillage of ROS into the cytoplasm can further aggravate damage to the various subcellular structures. Free radicals also cause suppression of DNA synthesis leading to loss of cell integrity and protein leakage.

Table 1

Group	Group A	Group B	Group C	Group D
Administration of	Saline	Vitamin C	Streptomycin	Streptomycin +Vitamin C
B. urea mg%	38.5 ± 2.2	41.6 ± 2.9	45.1 ± 1.3	39.4 ± 1.1
S. creatinine mg%	0.55 ± 0.12	0.63 ± 0.11	1.31 ± 0.13	0.61 ±0.12
Glutathione Peroxidase U/l	1031 ± 29	1204 ± 56	751 ± 48	845 ± 39
Glutathione Reductase U/l	74.2 ± 8.5	82 ± 3.7	46 ± 2.3	63 ± 2.8
Total Antioxidant Status mmol/l	1.31 ± 0.06	1.68 ± 0.03	1.11 ± 0.04	1.24 ± 0.04

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 [29]. In present study rise in level of blood urea and s. creatinine was suggestive of renal damage due to streptomycin.

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. *Ein vitro* studies have shown ability to generate free radicals and induce oxidative stress with aminoglycoside antibiotics [30]. In renal cortical mitochondria, formation of free radical like super oxide as well as hydrogen peroxide have been associated with aminoglycosides [31-33]. 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.

In addition to the intrinsic toxicity of an antibiotic, its nephrotoxicity depends upon physiological influences and pharmacokinetic considerations, such as the maximum concentration achieved and the duration of significant levels of the antibiotic. Pre-existing renal dysfunction, the disease process rendering the individual susceptible to infection, changes in renal blood flow, electrolyte imbalance and pre- or concomitant administration of other nephrotoxic drugs, increases the likelihood of deleterious effects of antibiotics on the kidney. These factors and the problem of distinguishing between the patient's underlying illness and drug induced nephrotoxicity make difficult the determination of the incidence of antibiotic-induced renal disease in patients. Such factors further complicate the determination of the mechanisms responsible for the deleterious action of antibiotics on the kidney.

Conclusion

The data generated from the present study concludes that

Antioxidant vitamin C has shown predictive benefit in streptomycin induced nephrotoxicity in recent study however more extensive studies are desirable. Free radicals are generated during treatment with streptomycin leading to nephrotoxicity which was significantly prevented by Coad ministration of vitamin C.

References

1. McWilliam SJ, Antoine DJ, Smyth RL, Pirmohamed M. Aminoglycoside-induced nephrotoxicity in children. Pediatr Nephrol. 2017; 32(11):2015-2025.
2. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011; 79(1):33-45.
3. Rougier F, Claude D, Maurin M, Maire P. Aminoglycoside nephrotoxicity. Curr Drug Targets Infect Disord. 2004; 4(2):153-62.
4. Plajer SM, Chin PK, Vella-Brincat JW, Buffery PJ, Begg EJ, *et al*. Gentamicin and renal function: lessons from 15 years' experience of a pharmacokinetic service for extended interval dosing of gentamicin. Ther Drug Monit. 2015; 37(1):98-103.
5. Biomarkers of nephrotoxic acute kidney injury. Ferguson MA, Vaidya VS, Bonventre JV Toxicology. 2008; 245(3):182-93.
6. Finn W, Porter G. Urinary biomarkers and nephrotoxicity. Clinical Nephrotoxins. 2nd ed Kluwer Academic Publishers; Massachusetts, 2003, 621-655.
7. Serum blood urea nitrogen as an independent marker of subsequent mortality among patients with acute coronary syndromes and normal to mildly reduced glomerular filtration rates.
8. Kirtane AJ, Leder DM, Waikar SS, Chertow GM, Ray KK, Pinto DS, *et al*. TIMI Study Group. J Am Coll Cardiol. 2005; 45(11):1781-6.
9. "Streptomycin Sulfate". The American Society of Health-System Pharmacists. Archived from the original on December 20, 2016. Retrieved, 2016.
10. Syal K, Srinivasan A, Banerjee D. "Streptomycin interference in Jaffe reaction Possible false positive creatinine estimation in excessive dose exposure". Clinical Biochemistry. 2013; 46(1-2):177-179. doi:10.1016/j.clinbiochem. 2012.10.031. PMID 23123914.
11. Sharma D, Cukras AR, Rogers EJ, Southworth DR, Green R. "Mutational analysis of S12 protein and implications for the accuracy of decoding by the ribosome". Journal of Molecular Biology. 2007; 374(4):1065-76. doi:10.1016/ j.jmb.2007.10.003. PMC 2200631. PMID 17967466.
12. Raymon Lionel P. COMLEX Level 1 Pharmacology Lecture Notes. Miami, FL: Kaplan, Inc, 2011, 181. CM4024K.
13. Voet Donald, Voet Judith G. Biochemistry (3rd ed.). John Wiley & Sons, 1994, 1341. ISBN 978-0-471-19350-0.
14. Jan-Thorsten Schantz, Kee-Woei Ng. A manual for primary human cell culture. World Scientific, 2004, 89.
15. Wainwright M. Miracle Cure: The Story of Penicillin and the Golden Age of Antibiotics. Blackwell. ISBN 9780631164920. Archived from the original on September 10, 2017. Retrieved, 1990.
16. Schatz, Albert. Bugle Elizabeth. Waksman Selman A. "Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria", Experimental Biology and Medicine. 1994; 55:66-69. doi:10.3181/00379727-55-14461.
17. Ryan, Frank. The forgotten plague: how the battle against tuberculosis was won-and lost. Boston: Little, Brown, 1993. ISBN 978-0316763806.
18. Cramer, Richard Ben. What It Takes New York, 1992, 110-11.
19. D'Arcy Hart P. "A change in scientific approach: from alternation to randomised allocation in clinical trials in the 1940s". BMJ. 1999; 319 (7209):572-3. doi:10.1136/bmj.319. 7209.572. PMC 1116443. PMID 10463905.
20. Wayner DD, Burton GW, Ingold KU. The antioxidant efficiency of vitamin C is concentration-dependent. Biochem Biophys Acta. 1986; 884:119-23
21. Padh H. Vitamin C: newer insights into its biochemical functions. Nutr Rev 1991; 49:65-70.
22. Maire PH, Corvaisier S, Rougier F, Bouvier D'Yvoire M, Claude D, Barbaut X, *et al*. Pharmacocinétique/pharmacodynamie clinique des antibiotiques. In Précis de bactériologie clinique. Editions ESKA, Paris, France, 2000,715-731
23. Kosek, JC, Mazze RI, Cousins J. Nephrotoxicity of gentamicin. Lab. Investig. 1974; 30:48-57.
24. Mingeot-Leclerq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob. Agents Chemother. 1999; 43:1003-1012.
25. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM. Gentamicin nephrotoxicity. I. Degree and permanence of acquired insensitivity. J. Lab. Clin. Med. 1982; 100:501-512.
26. Sahnoun Z, Jamoussi K, Zeghal KM. Free radicals and antioxidants: human physiology, pathology and therapeutic aspects. Therapie. 1997; 52:251-70
27. Hogg N. Free radicals in disease. Semin Reprod Endocrinol. 1998; 16:41-8.
28. Knight JA. Free radicals: their history and current status in aging and disease. Ann Clin Lab Sci. 1998; 28:331-46.
29. Fujita T, Fujimoto Y. Formation and removal of active oxygen species and lipid peroxides in biological systems. Nippon Yakurigaku Zasshi. 1992; 99:381-9.
30. Zima T, Tesar V, Stipek S, Nemecek K, Platenik J. The role of oxygen radicals in the pathogenesis of glomerulonephritis. Cas Lek Cesk. 1995; 134:716-9.
31. Sha SH, Schacht J. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res. 1999; 128:112-8
32. Yang CL, Du XH, Han YX. Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. Ren Fail. 1995; 17:21-6
33. Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest. 1988; 81:334-41
34. Sha SH, Schacht J. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res. 1999; 128:112-8.