Original Article

Nephrotoxicity in experimental rabbits and nephroprotective effect of metformin

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Objective: To evaluate potential role of metformin in protecting kidneys from nephrotoxic insult.

Methodology: Twenty four rabbits were randomly divided into four groups (n=6). G-1 received 1 ml isotonic saline intraperitoneally (IP) daily for 13 days. G-2 received daily gentamicin (40 mg/kg/day) IP for 13 days. G-3 received metformin salt (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days. G-4 received metformin salt (100 mg/kg/day) via feeding tube plus gentamicin (40 mg/kg/day) IP for 13 days. Blood was collected on days 0 and 14 for serum urea & creatinine estimation. All animals were sacrificed and kidneys were removed for renal histological examination.

Results: Metformin showed nephroprotective effect.

Conclusion: Metformin offers complete nephroprotection when administered along with nephrotoxic dose of gentamicin. This could offer an efficacious and cheaper treatment alternative in those diabetics who suffer from gram negative infections. (Rawal Med J 201;41:487-491)

Key Words: Nephrotoxicity, nephroprotection, renoprotection, gentamicin, metformin,

INTRODUCTION

The aminoglycoside gentamicin is widely used for treating gram negative infections. Unfortunately, about 10-20% of the patients may develop nephrotoxicity[1] and the oxidative stress induced by gentamicin plays key role in this nephrotoxicity.[2] The drug gets partially reabsorbed in renal proximal tubular cells and accumulates in lysosomes. This induces lysosomal phospholipidosis that in due course leads to gentamicin induced nephrotoxicity. The cytosolic gentamicin acts on mitochondria and produces oxidative stress, apoptosis and reduction of ATP reserve. Lack of ATP causes considerable proteolysis and necrosis.[3]

Gentamicin increases renal mitochondrial synthesis of reactive oxygen like hydroxyl radicals, hydrogen peroxides and superoxide anions, and nitrogen species, which consequently leads to functional as well as structural deterioration of kidneys.[4] This is reflected as deranged serum urea and creatinine; urinary losses of albumin and carnitine; reduced glomerular filtration rate,[8] desquamation of epithelial cells; glomerular congestion; atrophic and hypertrophic changes in glomeruli; perivascular and proximal tubular edema; and fibrotic and necrotic changes in tubules.[5]

The antidiabetic agent metformin, a biguanide, is used extensively for treatment of type 2 diabetes. It regulates glucose and fat metabolism in liver. The probable mechanisms involve the direct inhibition of gluconeogenic enzymes (e.g. phosphoenolpyruvate carboxykinase, fructose-1, 6-bisphosphatase and glucose-6-phosphatase), the reduced hepatic uptake of substrates for gluconeogenesis, and the increased phosphorylation of insulin receptor and insulin receptor substrates.[6]

Recent research has also shown metformin to exhibit antioxidant properties, complements mitochondrial function and thereby diminishes apoptosis induced by oxidative stress and appreciably decreases the depletion of respiratory components and prevents cell death.[7] It increases the thioredoxin expression, an antioxidant, through the AMP-activated protein kinase (AMPK) pathway which is responsible for reduction of ROS.

METHODOLOGY

The study was conducted in the department of Pharmacology, Army Medical College, Rawalpindi, Pakistan and included 24 healthy domestic rabbits of 7-10 months of age. Following drugs were used

Twenty four rabbits were randomly divided into four experimental groups, each group containing six rabbits. Gentamicin was given parenterally by intraperitoneal route.[8] Metformin was given by mouth mixed in drinking water via feeding tube. The drugs were given for 13 days following the underlying regime.[7,9] Injection gentamicin (containing 80 mg of gentamicin sulfate in 2 ml solution) from Reckitt & Coleman Pharmaceuticals, Karachi, Pakistan. Pure salt of Metformin donated by Werrick Pharmaceuticals, Islamabad, Pakistan

G-1 (Control group) was given isotonic saline in dose of 1 ml intraperitoneally once everyday[16] for 13 days. G-2 was given gentamicin injection intraperitoneally in nephrotoxic dose of 40 mg/kg/day for 13 days. G-3 received the antidiabetic drug metformin (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days. G-4 received injection of gentamicin (40 mg/kg/day) intraperitoneally plus metformin salt (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days.

Blood samples of rabbits were drawn twice during the study from the marginal ear vein i.e., on days 0 and 14. Urease/kinetic method[10] for serum urea and Jaffe reaction for serum creatinine[11] were used. All 24 rabbits were sacrificed on day 14 of study 24 hours after the administration of last doses of drugs. Kidneys were removed and processed for histopathologic examination. Tubular injury was graded as follows.[12] 0-No necrosis; 1- Minimal necrosis involving single cell in scattered tubules; 2-Moderate necrosis involving more than one cell in sparse tubules; 3-Marked necrosis with tubules showing complete necrosis in almost every single power field; and 4- Massive complete necrosis.

Results were assessed by Independent T Test and Paired T Test. 'Chi-Square Test' was used for histopathology results. $P<0.05$ was taken as statistically significant.

RESULTS

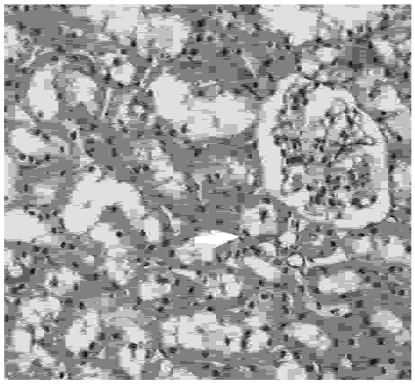
All the animals survived the fourteen days experimental period. Serum urea remained within the normal range over the entire experimental period in G-1 and G-3 rabbits (6.38±0.53 mmol/l on day 14). In comparison, serum urea increased to 12.71±1.31 mmol/l in G-2 ($P< 0.05$). The rabbits G-4 serum urea value was 6.96±0.09 mmol/l. When compared with G-2, the difference was statistically significant ($P<0.05$) (Table 1).

Serum creatinine also remained within normal range in G-1and G-3 over the experimental period. Serum creatinine was found significantly high on day 14 in animals of G-2 (140.33± 5.70 µmol/l) as compared to G-1 ($P<0.05$). In G-4, serum creatinine was 102.16±4.51 µmol/l. On comparison with G-2 (140.33±5.70 µmol/l), difference was significant ($P<0.05$) (Table 1).

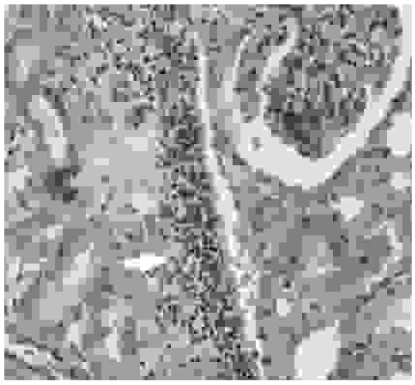
Table 1. Comparative analysis of serum parameters of different groups on day 0 and day 14.

Comparative Groups	Serum Urea (mmol/l)				Within Groups P value	Serum Creatinine (µmol/l)				Within Groups P value
	Day 0	SD	Day 14	SD		Day 0	SD	Day 14	SD	
G-1(normal saline)	6.96	0.41	6.38	1.30	0.298	104.33	6.08	104	8.85	0.910
G-2(gentamicin 40mg/kg/day)	6.38	0.99	12.71	3.22	0.002	107	10.17	140.33	13.96	0.006
Between Groups P value	0.001*					< 0.001*				
G-1(normal saline)	6.96	0.41	6.38	1.30	0.298	104.33	6.08	104	8.85	0.910
G-3(metformin 100mg/kg/day)	6.6	0.56	6.71	0.29	0.487	104.5	5.78	105	5.62	0.741
Between Groups P value	0.554Ns					0.820Ns				
G-2(gentamicin 40mg/kg/day)	6.38	0.99	12.71	3.22	0.002	107	10.17	140.33	13.96	0.006
G-4(metformin 100 mg/kg/day+ gentamicin 40 mg/kg/day)	6.88	0.31	6.96	0.24	0.363	99.16	9.64	102.16	11.05	0.380
Between Groups P value	0.001*					0.000*				

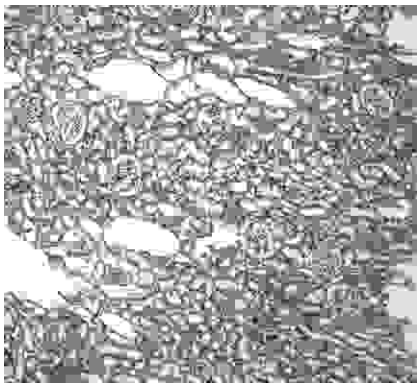
Fig. 1. Light micrographs showing histology of proximal renal tubules.



Normal histology of proximal renal tubules in rabbits of Gp-1 that received saline (400X).



Micrograph of Gp-2 treated with 40 mg/kg/day gentamicin showing inflammatory cells infiltration, congestion and tubular necrosis in renal tubules (400X)



Normal histology in sections of rabbits from G-4 treated with metformin and gentamicin revealing almost complete prevention of gentamicin induced histopathological alterations by biguanide (X100).

Histological exam of renal sections of G-1 animals was normal and categorized as grade 0 necrosis. In G-2, examination revealed moderate grade 2 necrosis in 67% of the animals. The remainder 33% rabbits exhibited mild grade 1 necrosis as evidenced by single cell necrosis in rare proximal tubules. There were also focal areas of degeneration of renal epithelial cell with or without evidence of desquamation. Examination of G-3 showed normal findings without any evidence of necrosis. Renal sections of G-4 were found to be normal with absence of signs of necrosis (Fig. 1). Chi square test was applied and the 'P' value was < 0.001, which showed the significant difference among the groups (Table 2).

Table 2. Comparison of histopathological findings of different groups,

Group	Grade-0 necrosis	Grade-1 necrosis	Grade-2 necrosis	Grade-3 necrosis	Grade-4 necrosis
1	+	---	---	---	---
2	---	---	+	---	---
3	+	---	---	---	---
4	+	---	---	---	---

P<0.001

DISCUSSION

In our study, the nephrotoxic group that was given 40 mg/kg/day of gentamicin showed deranged serum urea (P ? 0.002) and creatinine (P ? 0.001) values and grade 2 necrosis on renal histological exmination. Similar findings were observed by Chaware et al[13] and Yasin et al,[9] who also used gentamicin at a dose of 40 mg/kg/day for induction of nephrotoxicity in rats. Serum levels of urea and creatinine as well as histological findings were comparably deranged in these studies as were found in our study.

In our study, metformin has shown to possess nephroprotective potential. The animals which were administered gentamicin 40mg/kg/day along with metformin 100 mg/kg/day displayed complete nephroprotection. Their biochemical markers (serum urea and serum creatinine) as well as histological examination of their renal sections didn't show any alteration.

Lately, the recent research has brought metformin in lime light because of the discovery of its amazingly beneficial effects on cellular and mitochondrial functions.[14] It has been shown to increase intracellular antioxidant levels and consequently decrease reactive oxygen species significantly. A lot of work has been done to explore the diverse effects

of metformin and many researchers have confirmed its role in the prevention and management of diabetic nephropathy and protective effect against tubular cell injury.[15] It restores biochemical alterations in renal tubules.

This antidiabetic agent has been shown to remarkably reduce apoptosis induced by oxidative stress in the endothelial cells. Renoprotective potential of metformin has also been observed by many researchers.[16] Attenuation of diabetic nephropathy in rats by metformin through modulation of oxidative stress is reported by Alhaider et al.[17] In another pharmacological in-vitro study Hou et al[14] showed the inhibition of cell death and apoptosis by metformin. Morales et al[7] observed the similar antiapoptotic and antioxidant effects of metformin in the renal cells in an in-vitro study. In a study by Beeson et al[18] showed restoration of cell vitality in the cultured renal cells by metformin when they were exposed to nephrotoxicants like gentamicin. The results of the current study add further credibility to the nephroprotective effect of metformin.

Metformin offers complete nephroprotection against gentamicin induced nephrotoxicity that could prove particularly valuable in those diabetics who may suffer from gram negative infections. It could be an efficient treatment alternative in these patients, preventing the renal damage and maintaining the blood sugar at the same time.

CONCLUSION

Metformin when administered concomitantly with gentamicin prevented the antibiotic induced biochemical and histological kidney injuries.

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Conception and design: Ayesha Janjua, Salman Bakhtiar
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Analysis and interpretation of the data: Ayesha Janjua
Drafting of the article: Ayesha Janjua, Akbar Waheed
Critical revision of the article for important intellectual content: Ayesha Janjua, Akbar Waheed
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Conflict of Interest: None declared
Rec. Date: May 12, 2016 Revision Rec. Date: July 26, 2016 Accept Date: July 26, 2016

REFERENCES

1. Quiros Y, Vicente-Vicente L, Morales AI, Lopez-Nova JM, Lopez-Hernandez FJ. An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicol. Sci 2011;119:245-56.
2. Koyner JL, Sherali R, Murray PT. Antioxidants. Do they have a place in the prevention or therapy of acute kidney injury? Nephron Exp Nephrol 2008;109:109-17.
3. Al-Shabanah OA, Aleisa AM, Al-Yahya AA, Al-Rejaie SS, Bakheet SA, Fatani AG, et.al. Increased urinary losses of carnitine and decreased intramitochondrial coenzyme A in gentamicin-induced acute renal failure in rats. Nephrol Dial Transplant. 2010;25 :6976.
4. Balakumar P, Rohilla A, Thangathirupathi A. Gentamicin induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Pharmacol Res 2010;62:179-86.
5. Polat A, Parlakpinar H, Tasdemir S, Colak C, Vardi N, Ucar M. Protective role of aminoguanidine on gentamicin-induced acute renal failure in rats. Acta Histochem 2006;108:365-71.
6. Cheng JT, Huang CC, Liu IM, Tzeng TF, Chang CJ. Novel mechanism for plasma glucose-lowering action of metformin in streptozotocin-induced diabetic rats. Diabetes 2006;55:819-25.
7. Morales AI, Detaille D, Prieto M, Puente A, Briones E, Arévalo M, et al. Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. Kidney Int 2010;77:861-9.
8. Weir MR, Keniston RC, Enriquez JI Sr., McNamee GA. Depression of vitamin B6 levels due to gentamicin. Vet Hum Toxicol 1990;32:235-8.
9. Yasin KF, Sabic M, Khan-Sherwani M, Zafer M, Yasmin S, Alam M. Amelioration of gentamicin nephrotoxicity by vitamin B6 (a general histochemical profile). Pak J Med Res 2003;42:69-73.
10. Burtis CA, Ashwood ER. In: Tietz textbook of clinical chemistry. 3rd. Ed., W.B. Saunders Company, Philadelphla. 1999; p.1838.
11. Young DS. Effects of disease on clinical lab. In: Tests. 4th Ed. AACC. 2001.
12. Derakhshanfar A, Bidadkosh A, Sadeghian M. L-methionine attenuates gentamicin-induced nephrotoxicity in male wister rats: pathological and biochemical finding. Iranian J Vet Res 2009;10:323-8.
13. Chaware VJ, Chaudhary BP, Vaishnav MK, Biyani KR. Protective effect of the aqueous extract of Momordica charantia leaves on gentamicin induced nephrotoxicity in rats. Int J Pharm Tech Res 2011;3:553-5.
14. Hou X, Song J, Li XN, Zhang L, Wang X, Chen L, et al. Metformin reduces intracellular reactive oxygen species levels by upregulating expression of the antioxidant thioredoxin via the AMPK-FOXO3 pathway. Biochem Biophys Res Commun 2010;396:199-205.
15. Nasri H, Baradaran A, Ardalan MR, Mardani S, Momeni

A, Rafieian-Kopaei M. Bright renoprotective properties of metformin: beyond blood glucose regulatory effects. Iran J Kidney Dis 2013;7:423-8.
16. Piwkowska A, Rogacka D, Jankowski M, Dominiczak MH, Stepinski JK, Angielski S. Metformin induces suppression of NAD(P)H oxidase activity in podocytes. Biochem Biophys Res Commun 2010;393:268-73.
17. Alhaider AA, Korashy HM, Ahmed MMS, Mobark M, Kfoury H, Mansour MA. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression. Chem Biol Interact 2011;192:233-42.
18. Beeson CC, Beeson GC, Schnellmann R. A high-throughput respirometric assay for mitochondrial biogenesis and toxicity. Anal Biochem 2010;404:75-81.