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Effect of dimethyl sulfoxide in combat with gentamicin induced nephrotoxicity in rats

Afshin Hasanvand¹, Majid Tavafi^{2*}, Hassan Ahmadvand³, Ahmad Tamjidipoor²¹Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran²Department of Anatomy, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran³Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Introduction: Gentamicin sulphate (GS) induces nephrotoxicity by increasing of reactive oxygen species (ROS).

Objectives: The aim of this research was to assess the renoprotective effect of dimethyl sulfoxide (DMSO) as an antioxidant agent against GS-induced nephrotoxicity.

Materials and Methods: Forty male rats (Sprague-Dawley) were divided equally in to five groups and were treated as follows; group 1; received normal saline and served as normal controls, group 2; received GS (100 mg/kg/d), groups 3; received GS and DMSO (0.5 mL/kg/d), groups 4; received GS and DMSO (1 mL/kg/d), groups 5; received GS and DMSO (2 mL/kg/d). After eight days of treatment, serum was prepared. Paraffin sections (3 μ thickness) were prepared from the left kidneys and stained through periodic acid-Schiff (PAS) method. Serum creatinine and urea were assessed by the kits and serum malondialdehyde (MDA) was assessed by thiobarbituric acid (TBA) test. Volume density of proximal tubules, tubular necrosis, tubular cast and lymphocyte infiltration were evaluated histopathologically. The data were analyzed by Mann-Whitney U test at $P < 0.05$ by SPSS 12 software.

Results: Serum MDA, creatinine, urea, tubular volume density, tubular necrosis, tubular cast and lymphocyte infiltration were ameliorated significantly in groups four and five compared with group two ($P < 0.05$).

Conclusion: DMSO improved GS-induced renal toxicity significantly through prevention of lipid peroxidation and inflammation, but could not save kidney functional tests and histological changes at the same level as that of the normal group.

Implication for health policy/practice/research/medical education:

Nephrotoxicity due to gentamicin sulphate (GS) restricts its administration. Even though pathogenesis of GS-induced nephrotoxicity has not been understood entirely, recent investigations revealed that reactive oxygen species (ROS) participate in GS-induced nephrotoxicity. In this study dimethyl sulfoxide an antioxidant was used to inhibit GS-induced nephrotoxicity.

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Introduction

Gentamicin sulphate (GS) is usually administered to against gram-negative bacterial infections, but nephrotoxicity due to GS restricts its administration (1). Additionally, as an animal model GS-induced nephrotoxicity use for studying of nephrotoxic inhibitors in experimental researches.

Even though the pathogenesis of GS-induced nephrotoxicity has not been understood entirely, however, suggested mechanisms involved in GS-induced

nephrotoxicity includes; induction and progression of oxidative stress, apoptosis, necrosis and leucocyte infiltration (2,3). Gentamicin-induced nephrotoxicity characterized by increasing of serum creatinine and urea, reduction of glomerular filtration rate (4), disconnection of proximal convoluted tubule (PCT) cells from basal lamina and PCT cells death (5). Gentamicin induces high production of reactive oxygen species (ROS) such as superoxide anions (6), hydrogen peroxide, hydroxyl

*Corresponding author: Prof. Majid Tavafi, Ph.D,
Email: mtavafi@yahoo.com and tavafi.m@lums.ac.ir

radicals and reactive nitrogen species in the kidney (1). Reactive oxygen species stimulate nuclear factor kappa B (NFκβ) (7) and decline the kidney antioxidant enzymes activity like glutathione peroxidase (GPX) and catalase (3,8).

Objectives

Because of antioxidant and anti-inflammatory properties of DMSO (9-12) we decided to evaluate renoprotective effect of DMSO in rat model of gentamicin -induced nephrotoxicity.

Materials and Methods

Forty male Sprague-Dawley rats (135-150 gr) were divided randomly into five groups (eight per group). Group one received normal saline (1 mL/d) intraperitoneally (i.p.) and served as normal controls. Group two received GS only (100 mg/kg/d i.p.). Group three received GS (100 mg/kg/d i.p.) and DMSO (0.5 ml/kg/d i.p.). Group four received GS (100 mg/kg/d i.p.) and DMSO (1 ml/kg/d i.p.). Group five received GS (100 mg/kg/d i.p.) and DMSO (2 ml/kg/d i.p.). DMSO administrated one hour before GS injection for 8 days.

Animals were kept at 22°C and a moisture of 50 ± 10% with 12 hours of light/dark cycles. The animal's weights were calculated before and after the experiment (day 0 and day 8).

Twenty-four hours after the last GS injection, blood samples acquired from animal's hearts under anesthesia (sodium thiopental 50 mg/kg i.p.). After blood clotting in room temperature, the sera were prepared by centrifuging at 3000 rpm for 10 minutes. The animals' left kidneys were removed and fixed in 10% formalin solution. After 48 hours, fixed kidneys cut into one-millimeter thickness slices and after tissue processing, paraffin sections (3-μm thickness) were prepared and stained through periodic acid-Schiff (PAS) method.

Histopathological evaluations

PCT necrosis was evaluated semi-quantitatively on a score of 0 to 4 including zero, lacking cell necrosis; 1) one cell necrosis in scatter tubules; 2) more than one necrotic cells in scatter tubules; 3) cell necrosis in the most tubules and in every field and 4) total necrosis (13).

The tubular cast and leukocyte infiltration were evaluated using a semi-quantitative style as follows: zero, normal; 1, minimal; 2, mild; 3, moderate; 4, severe (1,14).

Volume density of PCT was estimated by point counting rule (14). Briefly, microscopic image was superimposed on a probe (frame 13 × 14-cm with 360 +). At ×300 linear total magnifications, the points that hit normal epithelium of PCT were counted. From each kidney, at least 60 microscopic fields were evaluated. The

volume density of PCT estimated through the following formula (15).

$PCT \text{ volume density} = \frac{\sum P_p}{\sum P_t}$ where $\sum P_p$ is the sum of points hitting normal PCT epithelium and $\sum P_t$ is points falling on reference space.

Biochemical analysis

Serum malondialdehyde (MDA) assessed by thiobarbituric acid (TBA) test. Serum creatinine and urea were determined through special kits (Ziest Chemie Diagnostic Company, Tehran, Iran) through its procedures. Serum sodium and potassium were assessed by flame photometer system.

Ethical issues

This study was approved by the Ethical Committee of Lorestan University of Medical Sciences (ethical code; IR.LUMS.REC.1392.261) that is in according to the ethical principles of the International Committees for the Protection of Animal Rights Laboratory and National Health and Medical Research Council guidelines.

Statistical analysis

The data were reported as mean ± SEM. The biochemical and histological data were analyzed through Mann-Whitney U-test. The animals and kidneys weight were analyzed through one-way ANOVA followed by Tukey test. All analysis was conducted by SPSS12 software and *P* value less than 0.05 was considered significant.

Results

Impact of DMSO on % body weight changes at day 0- day 8 and kidney weight

Gentamicin decreased the percentage of body weights changes in comparison with the normal group (*P* < 0.05). DMSO administration (2 mL/kg/d) improved the decline of percentage body weight change when compared to group 2 (*P* < 0.05, Table 1). GS increased the kidney weight in contrast to group 1 (*P* < 0.05) and treatment with DMSO (1 and 2 mL/kg/d) were kept kidney weight similar to normal group significantly (Table 1).

Serum parameters

Malondialdehyde was increased in sera of rats treated with GS in contrast to group 1 (*P* < 0.05). Treatment with DMSO decreased serum MDA significantly when compared with group 2 (Table 2). The level of serum creatinine increased in group 2 when compared with group 1 (*P* < 0.05; Table 2). Upon DMSO therapy in groups 4 and 5, a significant decrease (*P* < 0.05) in the levels of serum creatinine was seen in contrast to group 2 (Table 2).

Serum urea concentrations are shown in Table 2. GS

Table 1. Effect of DMSO on % body weight changes and kidney weight in rats against gentamicin sulphate

Groups	Pretreatment weight (g)	Post treatment weight (g)	% Body weight change (day 0-day 8)	Kidney weight (g) (day 8)
1. Normal	144.2±3.4	149/01±2.98	4.8±0.86	0.67±0.024
2. GS	167.62±9.18	160±9.02	-7.6±2.17*	0.88±0.052*
3. GS+DMSO (0.5 mL/kg/d)	145.87±10.48	139.62±9.01	-6.25±3.2*	0.79±0.023#*
4. GS+DMSO (1 mL/kg/d)	132.14±8.93	125±8	-7.14±1.3*	0.68±0.033#
5. GS+DMSO (2 mL/kg/d)	167.42±8.12	164.8±7.49	-2.57±1.49#*	0.67±0.038#

GS, gentamicin sulphate; DMSO, dimethyl sulfoxide.

Values represented as mean ± SEM.

* Significant change in comparison with normal group at $P < 0.05$. # Significant change in comparison with gentamicin only treated at $P < 0.05$.

Table 2. Effect of DMSO on serum parameters in rats against gentamicin sulphate

Groups	Serum creatinine(mg/dL)	Serum urea (mg/dL)	Serum malondialdehyde (nmol/mL)	Serum sodium(mmol/L)	Serum potassium (mmol/L)
1. Normal	0.43±0.06	38.52±10.1	0.23±0.01	173.2±9.94	4.98±0.31
2. GS	1.17±0.18*	103.8±18.6*	0.64±0.045*	187.8±8.32	6.53±0.035*
3. GS+DMSO (0.5 mL/kg/d)	1.1±0.11*	91.37±19.08*	0.51±0.048#*	174.9±12.88	5.6±0.38#
4. GS+DMSO (1 mL/kg/d)	0.78±0.11#*	66.37±15.8*#	0.501±0.038#*	166.5±14.32	4.85±0.35#
5. GS+DMSO (2 mL/kg/d)	0.81±0.12#*	67.5±13.1*#	0.49±0.033#*	173.5±10.17	4.01±0.21*#

GS, gentamicin sulphate; DMSO, dimethyl sulfoxide.

Values represented as mean ± SEM.

* Significant change in comparison with normal group at $P < 0.05$. # Significant change in comparison with gentamicin only treated at $P < 0.05$.

increased blood urea compared to group 1 ($P < 0.05$). Treatment with DMSO was considerably improved blood urea in groups 4 and 5 in comparison with group 2 ($P < 0.05$).

There is no significant difference between serum sodium concentration of experimental groups ($P < 0.05$).

GS increased serum potassium in group 2 when compared with the normal group ($P < 0.05$). In DMSO treated groups the serum potassium was saved at the same level as that of normal group ($P < 0.05$).

Histopathological results

Gentamicin administration in group 2 declined PCT volume density in contrast to group 1 ($P < 0.05$). DMSO therapy (1 and 2 mL/kg/d) improved PCT volume density significantly when compared with group 2 ($P < 0.05$), but could not keep the PCT volume density at the same level as that of group 1 (Table 3).

Gentamicin administration in group 2 increased tubular necrosis in contrast to group 1 significantly ($P < 0.05$) and DMSO therapy (1 and 2 mL/kg/d) attenuated the PCT necrosis when compared with group 2 ($P < 0.05$).

Table 3. Effect of DMSO on renal histopathological parameters in rats against gentamicin sulphate

Group	PCT volume density	Tubular necrosis (0-4)	Tubular cast (0-4)	Leucocyte infiltration (0-4)
1. Normal	0.22±0.08	0.24±0.04	0.16±0.016	0.42±0.2
2. GS	0.09±0.01*	1.91±0.29*	2.16±0.307*	3.33±0.33*
3. GS+DMSO(0.5ml/kg/d)	0.12±0.01*	1.38±0.24*	1.66±0.21*	2.83±0.31*
4. GS+DMSO(1ml/kg/d)	0.127±0.005#*	1.06±0.19#*	1.33±0.33#*	2.33±0.33#*
5. GS+DMSO(2ml/kg/d)	0.139±0.001#*	1.1±0.18#*	1.25±0.309#*	2.41±0.3#*

GS, gentamicin sulphate; DMSO, dimethyl sulfoxide.

Values represented as mean ± SEM.

* Significant change in comparison with normal group at $P < 0.05$. # Significant change in comparison with gentamicin only treated at $P < 0.05$.

GS administration in group 2 increased tubular cast significantly when compared with control group since treatment with DMSO (1 and 2mL/kg/d), reduced tubular cast compared with group 2 ($P < 0.05$) (Table 3).

Gentamicin administration in group 2 increased leucocyte infiltration significantly in comparison with group 1 while leucocyte infiltration was ameliorated by DMSO therapy (1 and 2mL/kg/d) in contrast to group 2 ($P < 0.05$).

Discussion

Reactive oxygen species (ROS) are the chief mediators in the pathogenesis of GS-induced nephrotoxicity (16,17).

DMSO showed antioxidant and anti-inflammatory properties in many studies (9-12). DMSO molecule has free electron pairs that makes it a powerful receptor of free radicals, especially hydroxyl radical (18).

In our research DMSO therapy improved body weight changes in combat with GS significantly. Such result was also reported by administration of aqueous leaf and seed extract of *Phyllanthus amarus* (19).

In our study kidney weight was increased significantly by GS similar to other reports (20,21). DMSO therapy saved the kidney weight at the same level as the normal group.

In this study, similar to other studies GS administration directed to significant increase in serum creatinine and urea (6,13,22-24). Treatment with DMSO improved serum creatinine and urea significantly in comparison with second group. Similar results were reported by using different antioxidants (17,23-25). Because of the tubular secretion of creatinine, it may be suggested that improvement of serum creatinine level by DMSO therapy take place probably via inhibition of tubular damages or activation of tubular cell regeneration. In early phases of the renal disorder, serum creatinine value is more important than the urea because serum urea increases after parenchymal injury (26).

In our research, serum MDA was increased by GS similar to other reports (27,28). Similar to our study, the other researchers reported improvement of serum MDA against GS nephrotoxicity with administration of different antioxidants (13,17,27,29). The elevated serum MDA shows intensification of free radicals production or lipid peroxidation. Gentamicin induces production of superoxide, H₂O₂ and hydroxyl radicals in mitochondria (16). Decrease of serum MDA following DMSO therapy in rat model of GS-induced nephrotoxicity may be due to inhibition of ROS or increase of renal tissue antioxidant enzymes. In addition, our results may be contributed to hydroxyl scavenging property of DMSO (18).

Based on our results and some reports (20,23), there is no significant difference between serum sodium

concentration between experimental groups. There are paradoxical results in serum sodium changes in GS-induced nephrotoxicity. Some reported increase of serum sodium and other reported decrease of sodium in GS treated animals (30,31).

In this investigation, similar to other reports (30,31) GS elevated serum potassium compared to the normal group. Administration of DMSO ameliorated concentration of serum potassium. Similar results were reported by administration of various antioxidants (30,31). However, there are some reports of hypokalemia in GS-induced nephrotoxicity (21).

Tubular necrosis ameliorated with DMSO therapy against GS nephrotoxicity. Similarly declining of PCT necrosis reported through various antioxidants administration (13,16). Gentamicin induces PCT cell death via oxidative stress, program cell death, and phospholipidosis (26). It has proposed that ROS plays a pivotal role in induction of renal tubular cell necrosis (7). Recovery of tubular necrosis with DMSO treatment may be due to inhibition of PCT cells death via ROS inhibition. After tubular cells necrosis, new tubular cells originate from differentiated mature cells that survived after GS injury. In this manner, various mature tubular cells dedifferentiate and then proliferate to produce new differentiated simple squamous tubular cells that cover bare area of injured tubules (32). Dedifferentiation and re-differentiation of mature tubular cell take place under influence of some factors such as hepatocyte growth factor, epidermal growth factor, insulin like growth factor-1, transforming growth factor beta and platelet derived growth factor (32). We propose that improvement of PCT necrosis with DMSO therapy is due to diminution of tubular cell death, activation of tubular cell regeneration or maintaining cell to cell and cell to basal lamina attachment.

Volume density of PCT reduced significantly in GS treated rats. The reduction of PCT volume density was improved through DMSO therapy against GS, but treatment cannot maintain it at the same level as that of the normal group. Absence of PCT brush border are the results of GS renal toxicity since DMSO maintained the brush border of PCT tubules too. Amelioration of PCT volume density by DMSO therapy may be contributed to inhibition of tubular necrosis, saving of apical membrane structural of PCT or new brush border formation after tubular cells regeneration.

Gentamicin induced renal lymphocyte infiltration while the administration of DMSO significantly reduced it. The same results were reported by administration of different antioxidants (3,6). Superoxide anions react with nitric oxide that makes peroxynitrite and then peroxynitrite activates NF κ B and finally NF κ B induces inflammation and lymphocyte infiltration (7). DMSO therapy reduced

probably lymphocyte infiltration via inhibition of superoxide or peroxy nitrite anions.

After GS administration, necrotic tubular cells and proteins excreted by tubular cells (Tamm–Horsfall protein) makes tubular cast in that maybe leads to tubular occlusion. In our study similar to other researches administration of different antioxidants reduced tubular cast significantly against GS nephrotoxicity (14,29).

In summary DMSO therapy reduced serum MDA significantly against GS nephrotoxicity. Functional renal factors (serum creatinine and urea) and histopathological variables (PCT volume density, tubular necrosis, tubular casts and lymphocyte infiltration) were ameliorated with administration of DMSO but did not save the variables at the same level as that of the normal values.

Although DMSO therapy attenuated the side effects of GS, treatments could not save tissue injuries and kidney functional tests as similar to the normal animals. Our findings suggested the beneficial effects of DMSO as a new nephroprotective agent against nephrotoxins like GS.

Conclusion

DMSO ameliorates significantly GS-induced nephrotoxicity probably via inhibition of lipid peroxidation and inflammation, but cannot save renal functional factors and histological changes at the same level as that of the normal values.

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Authors' contribution

AH and HA conducted the research. MT and AT designed and supervised the study, analyzed the data and prepared the final draft of the article.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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