doi:10.22100/ijhs.v3i3.268
Original Article

IJHS 2017;3(3):9-11
ijhs.shmu.ac.ir
IJHS
International Journal of Health Studies

Sulfur Dioxide Preserves Superoxide Dismutase and Catalase Activities in Acute Kidney Injury

Elham Biabanian[1], Mehdi Khaksari[1], Pirasteh Norouzi[1], Hossein Khastar[1*]
[1] School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.

Received: 8 October 2017
Accepted: 9 November 2017

Abstract

Background: *Acute kidney injury (AKI) is a major clinical problem in situations such as shock, sepsis, and kidney transplantation, and also occurs as a side effect of some drugs. Gentamicin (GM) is an effective antibiotic against severe gram-negative infections. However, it can produce AKI in humans. Reactive oxygen species (ROS) have been proposed as the causative factor for the renal side effects of GM. This study was performed to investigate the protective role of sulfur dioxide (SO_2) against GM-induced acute kidney injury in rats.*
Methods: *Male Wistar rats were randomly assigned to one of the following groups: 1, sham group; 2, GM group (100 mg/kg i.p. for 7 days); and 3, GM+SO2 group (5 μg/kg i.p. for 7 days). On day 8, renal tissues were collected for oxidative stress assessment. To compare the groups, superoxide dismutase (SOD) and catalase (CAT) in renal tissue were measured.*
Results: *GM caused significant acute kidney injury as demonstrated by the increase in BUN and creatinine levels in plasma. The decrease in renal tissue SOD and CAT levels revealed that oxidative stress occurred in the kidney. In the GM+SO_2 group, SO_2 prevented GM-induced reduction in SOD and CAT levels to some extent.*
Conclusions: *These findings suggest that SO_2 partly protects the kidneys from GM-induced nephrotoxicity by its antioxidant effect.*

Keywords: Gentamicin, Nephrotoxicity, Reactive oxygen species, Sulfur dioxide.
[*]**Corresponding to:** H Khastar, **Email:** h_khastar@yahoo.com
Please cite this paper as: Biabanian E, Khaksari M, Norouzi P, Khastar H. Sulfur dioxide preserves superoxide dismutase and catalase activities in acute kidney injury. Int J Health Stud 2017;3(3):9-11.

Introduction

Acute kidney injury (AKI) is a major clinical problem that occurs in some of the hospitalized patients, especially in intensive care units. Gentamicin (GM)-induced acute renal failure has been commonly used as a validated animal model to research acute kidney injury in experimental studies. Aminoglycoside antibiotics including gentamicin produce nephrotoxicity in humans.[1,2] These adverse renal effects may be induced by the generation of reactive oxygen species (ROS).[3] There are reports that antioxidant administration ameliorates GM-induced nephropathy.[4] Superoxide dismutase (SOD) and catalase (CAT) are the most important enzymatic antioxidant systems in the body.[5] SOD, as the first and most important line of defense against reactive oxygen metabolites (ROM), transforms superoxide ion to H_2O_2 that is a less reactive molecule.[5] CAT is a regulator of H_2O_2 metabolism and converts H_2O_2 into water. It is a tetrameric hemin-enzyme consisting of four identical tetrahedral arranged subunits.[6]

Sulphur dioxide (SO_2) is a sulfur-containing gas that is considered as a common air pollutant. Recently, it has been shown that SO_2 is a signaling molecule that is generated endogenously in mammalian tissues. It exerts significant biological effects such as antioxidant activity. Moreover, by administering SO_2 derivatives (bisulfite and sulfite), SO_2 is produced in mammalian body.[7,8] However, the biological effects of SO_2 are not completely understood.

Wang et al. found that pretreatment with a SO_2 donor (1–5 μmol/l) for 5 min before ischemia improved the recovery rate of LV± dp/dtmax and heart rate in isolated rat heart following ischemia/reperfusion (I/R) injury. This indicated that SO_2 pretreatment improved cardiac function in hearts with I/R injury in vitro.[9] In rats treated with a high cholesterol diet for 8 weeks, plasma total cholesterol increased, and high-density lipoprotein cholesterol decreased. After treatment with a SO_2 donor ($NaSO_3/NaHSO_3$, 0.54 mmol/kg: 0.18 mmol/kg injected intravenously daily), the plasma levels of triglyceride and low-density lipoprotein cholesterol were markedly decreased. In addition, the SO_2 donor significantly decreased atherosclerotic lesions.[10] Chen et al. reported that SO_2 has beneficial effects on lung injury by diminishing oxidative stress. Its administration profoundly reversed the elevated levels of OH- and H_2O_2 and significantly attenuated the malondialdehyde (MDA) levels in oleic acid-induced acute lung injury.[7] Jin et al. showed that apoptosis and the bcl-2/cytc/caspase-9/caspase-3 pathway in heart were inhibited by the intraperitoneal injections of SO_2 derivatives. For SO_2 administration, they used intraperitoneal injections of $Na_2SO_3/NaHSO_3$.[11,12]

In the present study, SOD and CAT levels of renal tissues, as important antioxidant indices, were assessed followed by GM-induced AKI. Then, effects of SO_2, as a novel antioxidant, were evaluated.

Materials and Methods

Male Sprague-Dawley rats (200 to 250 g) were maintained at room temperature (22 ℃±2 ℃) with a 12:12-h light–dark cycle and with free access to standard diet and water. Animal care was in compliance with the guidelines of the animal and human ethics committee of Shahroud University of medical sciences. Rats (N=27) were randomly assigned to 3 groups: 1, sham group; 2, GM group; and 3, GM+SO_2 group. In the sham group, 0.5 ml normal saline was injected intraperitoneally for 7 days. In the GM group, 100 mg/kg gentamicin in 0.5 ml normal saline was injected intraperitoneally for 7 days. Finally, in the GM+SO_2 group one hour before each GM injection, SO_2 (5 μg/kg) was injected intraperitoneally. The SO_2 group was administered an intraperitoneal injection of a SO_2 donor, $Na_2SO_3/NaHSO_3$, which was dissolved in 0.5 ml normal saline (1:3 M/M). On day 8, after general anesthesia, renal tissues of all animals were collected for oxidative stress assessment. Blood samples were obtained from the heart with heparinized syringes and centrifuged at 4000 g for 10 min at 4 °C. Plasma samples were collected for biochemical analysis. Plasma concentrations of blood urea nitrogen (BUN) and plasma Creatinine were evaluated by colorimetric methods using commercially available kits.

Renal SOD activity was determined by the method of Paoletti and Mocali.[13] In this method, superoxide anions are generated from oxygen molecules in the presence of $EDTAMnCl_2$ and mercaptoethanol. NAD (P) H oxidation is linked to the availability of superoxide anions in the medium. As soon as SOD is added to the assay mixture, it inhibits nucleotide oxidation. Therefore, at high concentration of the enzyme, the absorbance at 340 nm remains unchanged.

CAT activity was determined by Aebi's method.[6] According to this method, the activity of CAT can be measured by decomposition of H_2O_2. The remaining substrate concentration at a given moment of the reaction can be determined by UV spectrophotometry at 240 nm.

Results are expressed as means±standard error of mean (SEM). The statistical significance was determined by using one-way analysis of variance followed by Tukey's post-hoc test. Significant level was set at 0.05.

Results

Acute kidney damage was seen in the GM group with increased plasma BUN and Creatinine compared with the sham group (table 1).

Table 1. Biochemical parameters in groups

Biochemical parameters	Sham*	GM†	GM+SO_2‡
Plasma Creatinine, mg/dl	0.62±0.04	2.9±0.1 §	2.7±0.19 §
Plasma BUN, mg/dl	21±1.9	155±8.11 §	120±8.91 §||

*0.5 ml normal saline i.p.
†Gentamicin (100 mg/kg in 0.5 ml normal saline i.p.).
‡Sulphur dioxide (5 µg/kg i.p) +Gentamicin (100 mg/kg in 0.5 ml normal saline i.p.).
§Denotes P<0.01 compared with sham.
||Denotes P<0.05 compared with GM.

Intraperitoneal injection of GM (100 mg/kg) for 7 days led to decreases in renal SOD and CAT activities (figures 1 and 2).

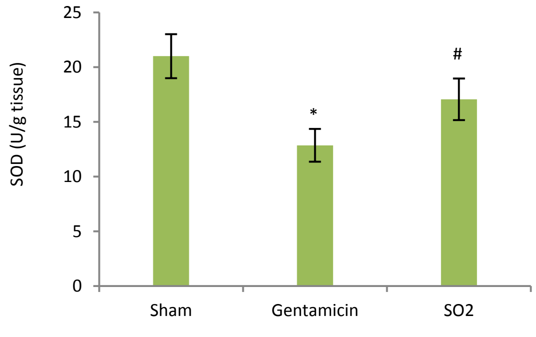

Figure 1. Renal SOD (mean±SEM)
***Denotes P<0.01 versus sham group; # Denotes P<0.05 versus (ischemia reperfusion) IR group**

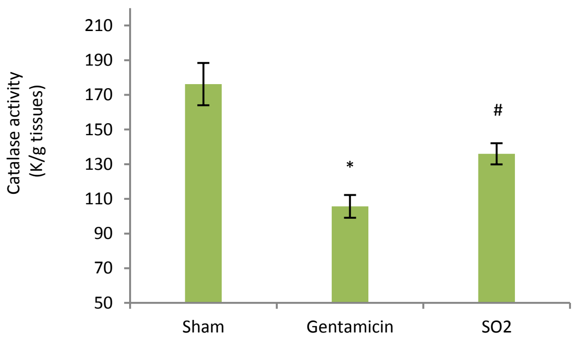

Figure 2. Renal CAT (mean±SEM)
***Denotes P<0.01 versus sham group; # Denotes P<0.05 versus IR group**

SO_2 injection at a dose of 5 µg/kg caused an increase in SOD and CAT activities in contrast with the sham group. Also, SO_2 diminished plasma BUN in this group in contrast with the GM group (table 1).

Discussion

GM-induced acute renal failure has been commonly used as a suitable animal model for AKI research.[14] GM is an aminoglycoside antibiotic which is used to treat severe gram-negative infections. In spite of the undesirable side effects such as hepatotoxicity, nephrotoxicity, and ototoxicity, GM is commonly used.[15]

In the present study, intraperitoneal injections of GM (100 mg/kg) for 7 days resulted in a decrease in plasma BUN and Creatinine that demonstrated acute renal failure. Moreover, SOD and CAT activity in the renal tissue of GM group decreased compared with the sham group. Some researchers have shown GM-induced oxidative stress with evaluation of oxidative stress indices. Otunctemur et al. reported that the glutathione levels in renal tissue of GM-treated rats were significantly lower than those in control group. In addition, the group given GM showed significantly higher MDA levels in the renal cortical tissue than control.[16]

Intraperitoneal injection of $Na_2SO_3/NaHSO_3$, a SO_2 donor which was dissolved in 0.5 ml normal saline (1:3 M/M), caused an increase in renal tissue SOD and CAT activity. The gasotransmitter SO_2 is a signaling molecule that was traditionally considered as an atmospheric pollutant.[17] SO_2 can be generated endogenously in mammalian tissues, and it exerts significant biological effects such as antioxidant activity.[8,11] It has recently been shown to be produced by mammalian cells.[12,17] Also, by administering SO_2 derivatives, $NaHSO_3/Na_2SO_3$ (1:3 M/M), SO_2 is produced in our body pH.

Chen et al. demonstrated that SO_2 has a protective effect on lung injury by inhibiting oxidative stress. MDA generation was significantly augmented in oleic acid-induced acute lung injury, but SO_2 significantly attenuated the MDA levels and inhibited lipid peroxidation. Moreover, antioxidant capacity was augmented by SO_2 administration. SO_2 administration significantly increased total antioxidant capacity and levels of catalase, SOD, and glutathione peroxidase (GPx) in oleic acid-treated rats.[7]

Liang et al. reported that SO_2 (intraperitoneal injection of 85 mg/kg for 7 continuous days) increased myocardial SOD, GPx levels, and messenger ribonucleic acid (mRNA) expression, and decreased H_2O_2 and ROS in isoproterenol-induced myocardial injury in rats.[18]

Hongfang et al. studied the regulation of mitochondrion-related cardiomyocyte apoptosis in rats with isopropylarterenol-induced myocardial injury and reported that apoptosis and the bcl-2/cytc/caspase-9/caspase-3 pathway in myocardial tissues were diminished by intraperitoneal injections of SO2 derivatives for 7 days.[12]

In rats with pulmonary hypertension induced by high pulmonary blood flow, SO_2 upregulated the endogenous H_2S pathway and improved pulmonary hypertension.[19] A mechanism that probably diminished the oxidative stress might be the upregulation of H_2S, a sulfur containing antioxidant, that limits cell damage and death.[20]

In conclusion, our results indicate that intraperitoneal injection of Na_2SO_3/$NaHSO_3$ as a SO_2 donor partly protected the kidneys from GM induced AKI by its antioxidant effect.

Acknowledgement

The authors would like to thank Shahroud University of Medical Sciences (Iran) for their support.

Conflict of Interest

The authors declared that they have no conflict of interest.

References

1. Chashmi NA, Emadi S, Khastar H. Protective effects of hydroxytyrosol on gentamicin induced nephrotoxicity in mice. Biochem Biophys Res Commun 2017;482:1427-9. doi:10.1016/j.bbrc.2016.12.052
2. Rafieian-Kopaei M, Baradaran A, Merrikhi A, Nematbakhsh M, Madihi Y, Nasri H. Efficacy of co-administration of garlic extract and metformin for prevention of gentamicin–renal toxicity in wistar rats: A biochemical study. Int j Prev Med 2013;4:258-64.
3. Kadkhodaee M, Khastar H, Faghihi M, Ghaznavi R, Zahmatkesh M. Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. Exp Physiol 2005;90:571-6. doi:10.1113/expphysiol.2004.029728
4. Mahmoud AM, Ahmed OM, Galaly SR. Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. EXCLI J 2014;13:98-110.
5. Kadkhodaee M, Khastar H, Arab HA, Ghaznavi R, Zahmatkesh M, Mahdavi-Mazdeh M. Antioxidant vitamins preserve superoxide dismutase activities in gentamicin-induced nephrotoxicity. Transplant proc 2007;39:864-5. doi:10.1016/j.transproceed.2007.02.038
6. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-6.
7. Chen S, Zheng S, Liu Z, Tang C, Zhao B, Du J, et al. Endogeous sulfur dioxide protects against oleic acid-induced acute lung injury in association with inhibition of oxidative stress in rats. Lab Invest 2015;95:142-56. doi:10.1038/labinvest.2014.147
8. Wang XB, Jin HF, Tang CS, Du JB. Significance of endogenous sulphur-containing gases in the cardiovascular system. Clin Exp Pharmacol Physiol 2010;37:745-52. doi:10.1111/j.1440-1681.2009.05249.x
9. Wang XB, Huang XM, Ochs T, Li XY, Jin HF, Tang CS, et al. Effect of sulfur dioxide preconditioning on rat myocardial ischemia/reperfusion injury by inducing endoplasmic reticulum stress. Basic Res Cardiol 2011;106:865-78. doi:10.1007/s00395-011-0176-x
10. Li W, Tang C, Jin H, Du J. Regulatory effects of sulfur dioxide on the development of atherosclerotic lesions and vascular hydrogen sulfide in atherosclerotic rats. Atherosclerosis 2011;215:323-30. doi:10.1016/j.atherosclerosis.2010.12.037
11. Luo L, Chen S, Jin H, Tang C, Du J. Endogenous generation of sulfur dioxide in rat tissues. Biochem Biophys Res Commun 2011;415:61-7. doi:10.1016/j.bbrc.2011.10.012
12. Jin H, Liu AD, Holmberg L, Zhao M, Chen S, Yang J, et al. The role of sulfur dioxide in the regulation of mitochondrion-related cardiomyocyte apoptosis in rats with isopropylarterenol-induced myocardial injury. Int j Mol Sci 2013;14:10465-82. doi:10.3390/ijms140510465
13. Paoletti F, Mocali A. Determination of superoxide dismutase activity by purely chemical system based on NAD (P) H Oxidation. Methods Enzymol 1990;186:209-20.
14. Tavafi M, Ahmadvand H, Toolabi P. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis 2012;6:25-32.
15. Sivachandran M, Hariharan P. Gentamicin induced oxidative stress on renal antioxidant parameters and its ameiloration by Andrographis paniculata in rats. Int J Agricul Biosci 2012;1:11-5.
16. Otunctemur A, Ozbek E, Cekmen M, Cakir SS, Dursun M, Polat EC, et al. Protective effect of montelukast which is cysteinyl-leukotriene receptor antagonist on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Ren fail 2013;35:403-10. doi:10.3109/0886022X.2012.761040
17. Wang XB, Du JB, Cui H. Sulfur dioxide, a double-faced molecule in mammals. Life Sci 2014;98:63-7. doi:10.1016/j.lfs.2013.12.027
18. Liang Y, Liu D, Ochs T, Tang C, Chen S, Zhang S, et al. Endogenous sulfur dioxide protects against isoproterenol-induced myocardial injury and increases myocardial antioxidant capacity in rats. Lab Invest 2011;91:12-23. doi:10.1038/labinvest.2010.156
19. Luo L, Liu D, Tang C, Du J, Liu AD, Holmberg L, et al. Sulfur dioxide upregulates the inhibited endogenous hydrogen sulfide pathway in rats with pulmonary hypertension induced by high pulmonary blood flow. Bioche Biophys Res Commun 2013;433:519-25. doi:10.1016/j.bbrc.2013.03.014
20. Polhemus DJ, Calvert JW, Butler J, Lefer DJ. The cardioprotective actions of hydrogen sulfide in acute myocardial infarction and heart failure. Scientifica (Cairo) 2014;2014:1-8. doi:10.1155/2014/768607