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Original Article

Improving Effect of Aminoguanidine on Lipopolysaccharide-Caused Kidney Dysfunction in Rats

Mahmoud Hosseini[1], Farimah Beheshti[2,3], Akbar Anaeigoudari[4]

[1]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, [2]Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran, [3]Department of Physiology, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran, [4]Department of Physiology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran

ABSTRACT. Kidneys have been shown to be the main target for toxins. Lipopolysaccharide (LPS) is a bacterial endotoxin which can involve in pathogenesis of endotoxemia-caused kidney dysfunction. Excessive production of free radicals such as nitric oxide (NO) and pro-inflammatory cytokines have been reported to contribute in kidney dysfunction. The purpose of this study was to investigate the effect of inducible nitric oxide synthase (iNOS) inhibition against LPS-induced kidney dysfunction in rats. Male rats were assigned into five groups. Control animals were injected saline; LPS group received 1 mg/kg of LPS for five weeks; LPS-AG50, LPS-AG100, and LPS-AG150 groups received AG (50, 100, and 150 mg/kg, respectively) 30 min before LPS. All drugs were administered intraperitoneally. LPS injection enhanced the level of blood urea nitrogen (BUN) and creatinine compared with the control group. Pretreatment with AG resulted in a significant reduction in BUN and creatinine in LPS-AG100 and LPS-AG 150 groups with respect to the LPS group. LPS administration led to a significant increase in interleukin (IL)-6, malondialdehyde (MDA), and NO metabolites as well as a significant decrease in the content of total thiol groups and superoxide dismutase (SOD) and catalase (CAT) activity. Pretreatment with AG reduced the level of IL-6, MDA, and NO metabolites and enhanced the content of total thiol groups and SOD and CAT activity in LPS-AG groups compared to the LPS group. The results of the present study show that inhibition of iNOS has a protective effect against kidney dysfunction caused by LPS.

Correspondence to:

Dr. Akbar Anaeigoudari,
Department of Physiology,
School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran.
E-mail: Anaeiga317@gmail.com

Introduction

Kidneys have been shown to be as a main target for toxins.[1] Endotoxemia, which take place under pathological conditions, including infection plays an important role in kidney injury.[2] It has been reported that lipopolysa-

ccharide (LPS), a bacterial endotoxin, can involve in the pathogenesis of endotoxemia-caused kidney dysfunction.[3] It has been also suggested that kidney dysfunction can associate with overproduction free radicals, reduction glutathione (GSH) levels, and excessive generation of inflammatory cytokines.[4] There are numerous studies confirming the role of LPS in promoting the overproduction of free radicals and pro-inflammatory cytokines.[5,6] For example, LPS has been revealed to stimulate the excessive generation of nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6).[7] In addition, the several signaling pathways, including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and nuclear factor κB (NFκB) involve in LPS-stimulated inflammation and oxidative stress.[7,8] Given these signaling pathways involving in induction inflammatory responses and oxidative stress, the researchers would like to find anti-inflammatory and antioxidant agents that inhibit the overproduction of inflammatory and oxidative damage mediators by LPS.

It has been reported that inhibition of iNOS could be associated with attenuating inflammatory responses and oxidative stress.[9] Amioguanidine (AG), an iNOS inhibitor, has been reported to have anti-inflammatory[10] and antioxidant[11] effects. Scientific evidence show that AG reduces the expression of pro-inflammatory cytokines involved in chronic inflammatory responses including IL-1β and TNF-α.[10,12] In addition AG has been revealed to protect tissues against oxidative damage by diminishing NO production and enhancing GSH content.[13] Previously in a study, the researchers also suggested that AG can improve cisplatin-caused nephrotoxicity due to its antioxidant properties.[14] Considering these reports, the current study was aimed to study the effect of iNOS inhibition by AG against LPS-induced kidney dysfunction in rat.

Materials and Methods

The current study was conducted on forty male Wistar rats. The rats were randomly grouped into five groups (n = 8 rats/group) and treated for five weeks as follows: control group was injected with normal saline. LPS group was administered with LPS (1 mg/kg). LPS-AG50, LPS-AG100, and LPS-AG150 groups were injected with AG (50, 100, and 150 mg/kg, respectively) 30 min before LPS. The animals of the LPS group were administered saline 30 min before LPS instead of AG. Drugs were dissolved in saline and were injected intraperitoneally. All experimental protocols were carried out in accordance with procedures approved by the Committee on Animal Research of Mashhad University of Medical Sciences (Process Number 9000559). LPS (*Escherichia coli* 055-B5) and AG were purchased from Sigma (Sigma Aldrich Chemical Co.). Other chemicals used to do biochemical assessments were purchased from Merck Company.

Blood sample and kidney tissue collection

At the end of the experimental period (5 weeks), the animals were anesthetized with urethan. Blood samples were collected from the heart and centrifuged at 3500 rpm for 10 min for collection sera. Sera were kept at -80°C until measurement of blood urea nitrogen (BUN) and creatinine. Then the animals were scarified and their kidneys were omitted to measure the IL-6 concentration and biochemical parameters.

Determination of blood urea nitrogen and creatinine

Serum levels of BUN and creatinine were determined by enzymatic colorimetric kits (Pars Azmoon Company, Tehran, Iran) according to the protocol provided by the company.

Determination of interluekin-6

The level of IL-6 in kidney tissue was determined by an ELISA kit (Ebioscience Co., San Diego, CA, USA) in accordance with the manufacturer's protocol.

Determination of oxidative stress parameters in kidney tissue [malondialdehyde, total thiol group (glutathione), nitric oxide, superoxide dismutase *and catalase]*

Kidney tissue MDA concentration was determined by a spectrophotometric method. In this method, the reaction of MDA with thiobarbituric acid leads to the formation of a colored complex which its peak absorbance is at 535 nm.

GSH content in kidney tissue was evaluated by a spectrophotometric method based on sulfhydryl of GSH reaction with 2,2′-dinitro-5,5′ dithiodibenzoic acid. Sulfhydryl reaction with 2,2′-dinitro-5,5′ dithiodibenzoic acid produces a yellow colored complex with a peak absorbance at 535 nm.

Level of total nitrite in kidney tissue was estimated by the Griess method. In this method, nitrate was reduced to nitrite, which forms a color appearance with Griess reagent in acidic medium.

Kidney activity of SOD was measured by a spectrophotometric method. The method is based on the autoxidation of pyrogallol which was inhibited by SOD. SOD activity was estimated at 570 nm. One unit of SOD was expressed as the amount of enzyme that inhibits % 50 rate of autoxidation of pyrogallol.

The activity of CAT in kidney tissue was estimated using the Aebi's method. In this method, H_2O_2 as substrate of CAT is broken into water and oxygen that shows itself on the ultraviolet spectrum as a reduction in the absorbance.

Statistical Analysis

Results were showed as means ± standard error of mean. Statistical comparison has been carried out by one-way ANOVA followed by Tukey's test. Differences with P <0.05 were considered significant.

Results

Effect of amioguanidine on kidney function

To determine the protective effect of AG on kidney function, serum levels of BUN and creatinine were measured. LPS administration resulted in a significant increase in the level of BUN and creatinine compared with the control group (P <0.001). AG significantly reduced the serum levels of BUN and creatinine in LPS-AG100 and LPS-AG 150 groups when compared with the LPS group (P <0.01 and <0.001) (Figure 1a and b).

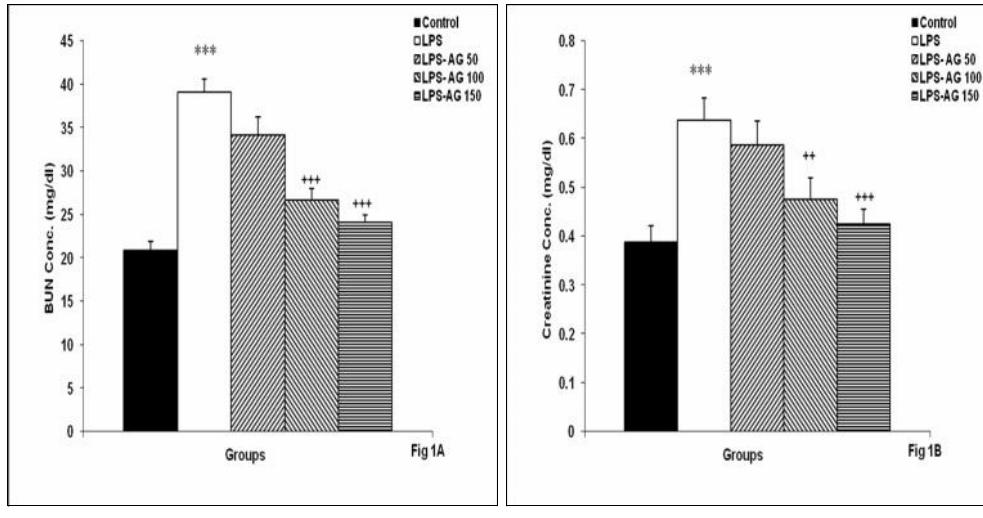


Figure 1. (a and b) Comparison of the serum level of blood urea nitrogen and creatinine between groups. Data are shown as mean ± standard error of mean (n = 8 rats/ group).

***P <0.001 compared with control group. ^{++}P <0.01 and ^{+++}P <0.001 compared with lipopolysaccharide group.

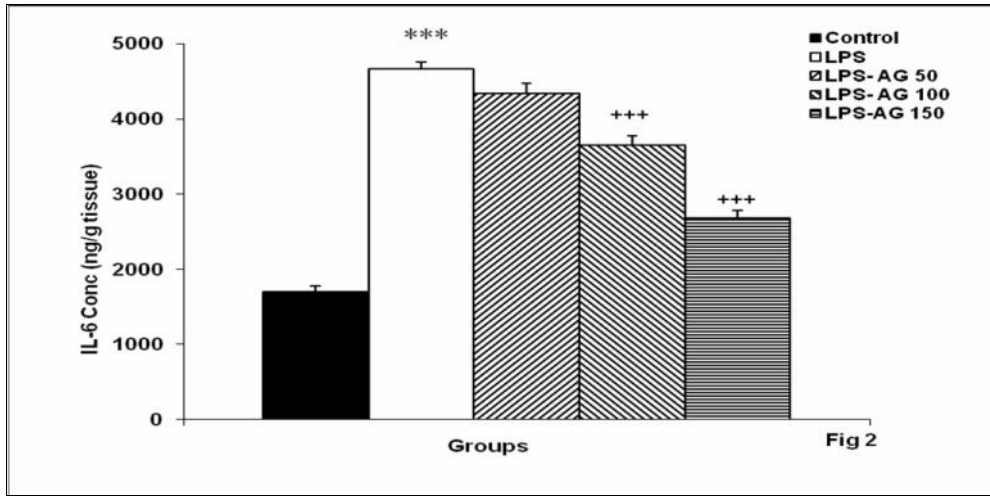


Figure 2. Comparison of the interleukin-6 concentration in kidney tissue between groups. Data are shown as mean ± standard error of mean (n = 8 rats/ group).

***P <0.01 compared with control group. ^{+++}P <0.01 compared with lipopolysaccharide group.

Effect of amioguanidine on concentration of interleukin-6 in kidney tissue

To study the effect of AG on LPS-induced inflammation in the kidney, the level of IL-6 in kidney tissues was measured. As shown inFigure 2, LPS injection markedly enhanced the IL-6 concentration in LPS- treated animals compared with the control group (P <0.001). Administration of AG before LPS decreased the level of IL-6 in LPS-AG100 and LPS-AG150 groups compared with LPS group (P <0.001).

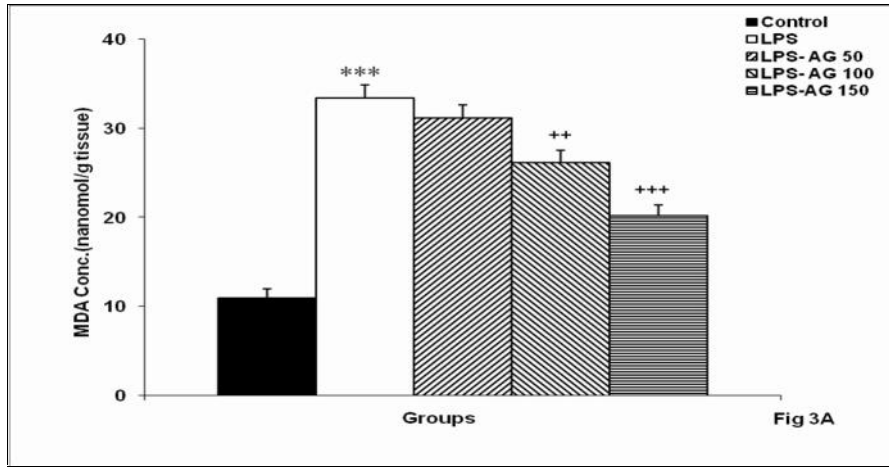
Effect of AG on oxidative stress parameters in kidney tissue

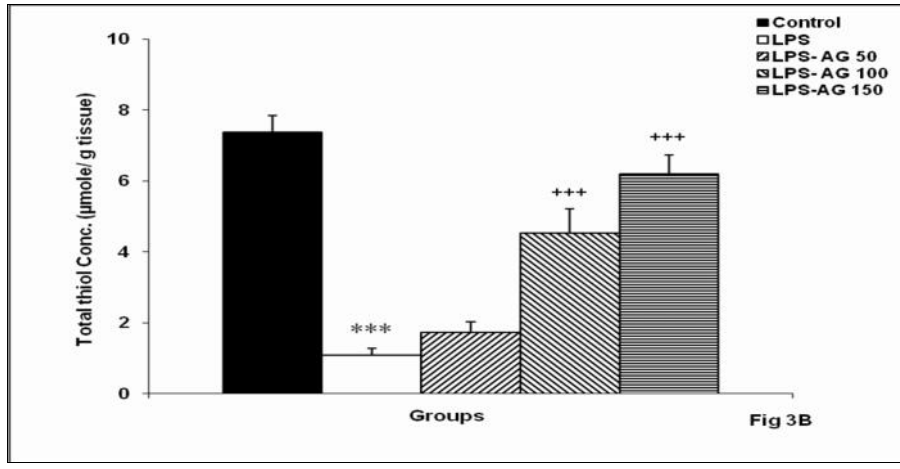
In this study, we evaluated the effect of AG on LPS-induced oxidative stress in kidney tissues. LPS caused a significant increase in MDA and NO metabolites concentration as well as a significant decrease in total thiol content in LPS group compared with the control group (P <0.001). Injection of AG significantly decreased MDA and NO metabolites concentration and enhanced the total thion content in LPS-AG100 and LPS-AG150 groups with respect to LPS group (P <0.01 and P <0.001) (Figure 3a-c).

In addition, LPS caused a significant decrease in CAT and SOD activity in LPS group compared with control group (P <0.001). AG-treated rats revealed a significant increase in CAT and SOD activity in LPS-AG100 and LPS-AG 150 groups compared to LPS group (P <0.001) (Figure 4a and b).

Discussion

The result of the present study provides evidence to support that inhibition of iNOS by AG attenuates the detrimental effects of LPS on kidney in rat. Endotoxemia has been shown to play a basic role in kidney injury.[2] It has been reported that contamination with bacterial endotoxins such LPS can result in kidney dysfunction.[15] Kidney dysfunction is characterized by a reduction in glomerular filtration rate resulting in an enhancement in serum level BUN and creatinine.[16] In this study, we manifested the effect of LPS administration on kidney function by measuring the serum level of BUN and creatinine in rats. According to the results of the current study, the animals injected with LPS had worse kidney function than untreated animals. This finding was confirmed by increased serum levels of BUN and creatinine in the LPS group when compared with the control group. Based on the previous studies, kidney dysfunction





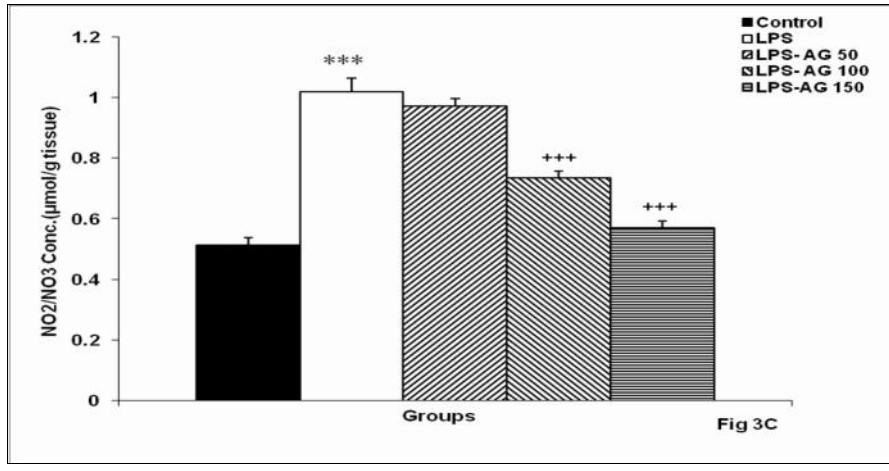
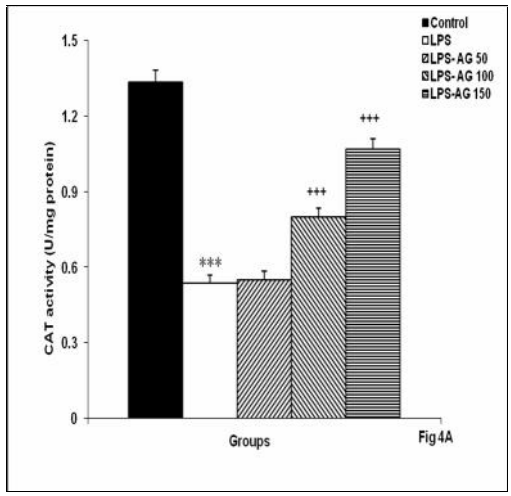


Figure 3. Comparison of the malondialdehyde concentration (a), the total thiol concentration (b) and the level of nitric oxide metabolites (c) in kidney tissue of five groups. Data are presented as Mean ± standard error of mean (n = 8 rat/ group).

***P <0.001 compared with control group. ^{++}P <0.01 and ^{+++}P <0.001 compared with lipopolysaccharide group.



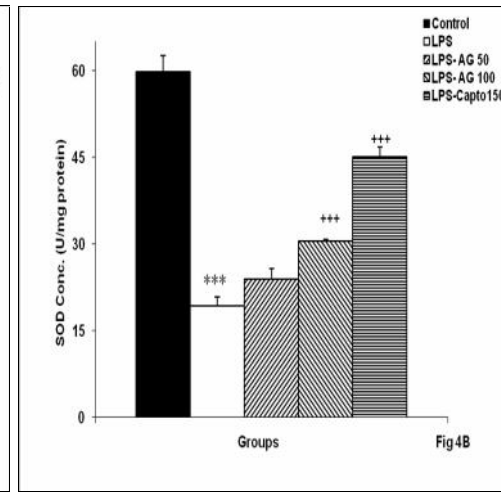


Figure 4. Comparison the activity of catalase (a) and superoxide dismutase (b) in kidney tissue between five groups. Data are shown as mean ± standard error of mean (n = 8 in each group).

***P <0.001 compared with control group and ^{+++}P <0.001compared with lipopolysaccharide group.

could be a result of the uncontrolled production of pro-inflammatory cytokines.[4] For example nephropathy has been shown to take place after high generation of inflammatory cytokines such as IL-1, IL-6 and IL-33.[17] In addition, some IL-1 family cytokines including IL-33 has been reported to have a basic role in the pathogenesis of some experimental models of kidney damage including kidney ischemia-reperfusion,[18] cisplatin-caused kidney injury[19] and oval bumin-induced nephrotoxicity models.[20] There also are evidence of LPS effect on excessive production of inflammatory cytokines[21,22] and free radicals, including NO[23] by immune cells. In addition, the researchers demonstrated the suppression of inflammatory responses by IL-1β inhibitors such as diacerein improved LPS- induced kidney dysfunction in mice through decreasing the level of IL-1β and TNF-α.[2] In line with these reports, in our study, LPS injection resulted in a significant enhancement in the level of IL-6 in kidney tissue in LPS group compared with the control group.

Another cause considered in the pathogenesis of kidney dysfunction is oxidative stress. The harmful effect of lipid peroxidase on the glomerular basement membrane has well been documented.[24] Overproduction of reactive oxygen species and oxidative damage has also been demonstrated to involve in kidney damage in several experimental models such as sepsis, severe burns, toxins, and ischemia reperfusion.[25] In addition, the result of our previous studies determined that LPS administration disturbed oxidative balance through increased concentration of MDA and reduced content of total thiol groups in rat.[26] In present study LPS injection also attenuated oxidative status in kidney tissue. To prove this finding LPS administration resulted in a significant increase in the level of MDA and NO metabolites as well as a significant decrease in content total thiol groups and SOD and CAT activity in kidney tissue in LPS- treated animals with respect to untreated rats.

In this study, we observed that inhibition of iNOS by AG restored the kidney dysfunction caused by LPS in rats. In supporting this claim, the animal treated with AG has better kidney function that those of LPS treated. In this study the serum level of BUN and creatinine in LPS- AG 100 and LPS-AG150 was lower than the LPS group.

It has been proposed that iNOS inhibition could be associated with improving inflammatory status.[27] On the other hand contribution of LPS, IL-1 and TNF-α in induction of iNOS in

pathophysiology of some organs such as the liver was documented.[28] AG as an selective inhibitor of iNOS has been shown to have anti-inflammatory effects.[29] Decreased levels of TNF-α, IL-8, and IL-1α by AG have been documented.[30] In addition, it has been indicated that AG ameliorated kidney injury in multiple kidney diseases such as lupus nephropathy, experimental diabetic nephropathy due to its effects in inhibition of inflammatory responses.[31] These findings are in consistence with the results of the current study that reported a decrease in inflammatory responses accompanied with a reduction in the content of IL-6 in AG groups compared to LPS group in kidney tissue.

Researchers also revealed that iNOS expressed in response to bacterial pathogens involve in the overproduction of free radical NO.[32] It was well known that NO could react with other reactive oxygen species such as superoxide, which is associated with oxidative stress.[9] NO has been suggested to have serious harmful effects on various organs due to its effects on overexpression of pro-inflammatory cytokines and induction of oxidative stress.[33] In addition, it has been shown that AG protects tissues against oxidative stress by decreasing NO.[34] AG has been suggested to ameliorate cyclophosphamide – induced oxidative stress in rat.[13] It has been also reported that AG improved nephrotoxicity caused by cisplatin due to its antioxidant properties.[14] In addition, AG has been revealed to have useful effects on radiation-caused lung toxicity via reducing oxidative stress in the rat.[35] In agreement with these findings, in the present study AG administration resulted in a significant reduction in MDA and NO metabolites as well as a significant enhancement in total thiol groups content and CAT and SOD activities in kidney tissue in LPS-AG groups with respect to LPS group. Considering these data, it seems that the ameliorating effect of AG against LPS – induced kidney dysfunction might be mediated due to its useful effects on inflammatory responses and oxidative stress caused by LPS. However, the exact mechanism need to be more studied. In summary, these results showed that iNOS inhibition with AG has a protective effect against kidney dysfunction caused by LPS. According the results of the present study protective effect of AG is likely mediated through decreasing inflammatory responses and oxidative stress induced by LPS.

Acknowledgments

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Conflict of interest: None declared.

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