Original Article

The effect of an specific inducible NO synthase inhibitor, S-methylisothiourea hemisulfate on cisplatin-induced nephrotoxicity; gender-related differences

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Abstract

Backgrounds: It has been previously demonstrated that the increase of nitric oxide (NO) level may promote cisplatin (CP)-induced nephrotoxicity. The aim of this study was to investigate the role of inducible NO synthase (iNOS) inhibitor to prevent CP-induced nephrotoxicity.

Materials and Methods: Four groups of male and four groups of female rats were treated daily with vehicle, S-methylisothiourea hemisulfate (SMT) as a selective iNOS inhibitor (5 mg/kg/twice a day), CP (2.5 mg/kg/day), and CP + SMT for 6 days. Then, all animals were sacrificed and the serum levels of creatinine (Cr), blood urea nitrogen (BUN), nitrite, and malondialdehyde (MDA) were measured. The kidney was removed immediately for histopathological study.

Results: Our results showed that inhibition of iNOS by SMT could make different response in male and female animals. SMT therapy in male animals decreased serum BUN, Cr, nitrite, and MDA levels; and it also protected kidney against CP-induced nephrotoxicity.

Conclusion: It is concluded that decrease in NO production by SMT has a beneficial effect on reducing CP-induced nephrotoxicity in male. However, such beneficial effect was not observed in female animals.

Key Words: Cisplatin, nephrotoxicity, nitric oxide, SMT

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Received: 19.06.2014, **Accepted:** 04.08.2014

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II; CP) is an effective antineoplastic agent in treatment of various solid tumours including cancers of the ovary, testis, bladder, head, neck, lung, cervix, and endometrium.[1,2] Nevertheless, the full clinical utility of the agent is limited due to some adverse side effects including renal toxicity. The major site of renal injury is the S3 segment of the proximal tubule.[3] CP may disturb endothelium and endothelial function,[3] which recognized by nitric oxide (NO) as a marker of endothelial function. It has been found that the renal content of total nitrate/nitrite is increased in CP-treated rats.[4,5] This is while increase in NO level may promote CP-induced

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.161223

How to cite this article: Ghayyoomi M, Soltani N, Nematbakhsh M, Moslemi F, Talebi A, Shirdavani S, *et al.* The effect of an specific inducible NO synthase inhibitor, S-methylisothiourea hemisulfate on cisplatin-induced nephrotoxicity; gender-related differences. Adv Biomed Res 2015;4:130.

nephrotoxicity.[4] Furthermore, the inhibition of NOS by aminoguanidine[6] decreases renal damage induced by CP, suggesting that NO plays a toxic role in the experimental model while it has shown that vitamin E could reduce CP-induced nephrotoxicity via decrease in the level of all NO metabolites.[7]

On the other hand, it is documented that CP induces oxidative stress,[8] and the induced nephrotoxicity is gender related.[9-11] Accordingly the induced nephrotoxicity intensity in males is more than in females.

NO is synthesized from the amino acid l-arginine by the endothelial NO synthase (eNOS). It is documented that l-arginine, as the precursor of NO, has a protective role against CP-induced nephrotoxicity.[8,12] Our previous finding[12] demonstrated that l-arginine may be protective against CP-induced nephrotoxicity in males, but it promotes the induced damage in females. There is some evidence that NO release could be gender-related.[13] We also found that l-arginine and losartan provide their nephroprotectant effect against CP-induced nephrotoxicity by reducing the serum level of nitrite.[14] Some paradoxes could be seen here; CP may increase NO,[12,15] NO may promote CP-induced nephrotoxicity,[4] and NO may donates some agents[16] which possibly attenuate CP-induced kidney toxicity. To answer this paradox, one should define which type of NOS expression is changed during CP therapy. Accordingly, this study was designed to investigate the role of iNOS inhibitor (S-methylisothiourea hemisulfate, SMT) to prevent CP-induced nephrotoxicity.

MATERIALS AND METHODS

Animal

The animal protocol of this study was in advance approved by Isfahan University of Medical Sciences Ethics Committee. Male and female Wistar rats, weighing 180-250 g were chosen and kept at a constant temperature of 22 ± 2ºC with a fixed 12:12-h light-dark cycle. Nutritionally balanced pellets and water were freely available. The animals were divided into eight groups (7 rats in each group) and treated for six continuous days as follows: Male intact control (sham), female intact control (sham), male control received SMT "a selective iNOS inhibitor" 5 mg/kg/twice a day via ip injection, female control received SMT, male CP-treated received 2.5 mg/kg/day of CP (9) by i.p. injection, female CP treated, male and female treated with CP + SMT. Bodyweight of all animals was recorded daily using a digital scale.

Six days later, all animals were anesthetized by ketamine HCl (75 mg/kg), and blood samples were taken and the rats were sacrificed. The kidney was removed immediately for histopathological study.

Biochemical assay

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were determined using quantitative diagnostic kits (Pars Azmoon, Tehran, Iran). The serum level of nitrite was measured using a colorimetric assay that involves the Griess reaction. The serum level of malondialdehyde (MDA) was measured by manual method. Briefly, 0.5 mL of the sample was mixed with 1 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 2000 *g* for 10 min. Then, 500 μL of the supernatant was added to 500 μL of 0.67% thiobarbituric acid (TBA) and was incubated in the boiling water for 10 min. After cooling down, the absorbance was read at the wavelength of 532 nm.

Histopathological procedures

The kidney damage induced by CP was scored by an expert pathologist unaware of the animal groups. The removed kidney was weighted and fixed in 10% neutral formalin solution and embedded in paraffin for histopathological staining. The staining was applied through H and E staining to examine the tubular damage. To score the induced damage from 1 to 4, the presence of tubular atrophy, cast, debris, and necrotic material in the tubular lumen and lymphocytes in interstitial tissue were considered, and zero was assigned to normal tissue.

Drugs

The following drugs were used: CP was purchased from EBEWE Pharma GES.m.b.H (Unterach, Austria), SMT was obtained from Sigma (St. Louis, MO, USA), and ketamine HCl was obtained from Rotexmedica (Trittau, Germany).

Statistical analysis

Data were expressed as mean ± SE.M. One-way ANOVA with the Tukey *post hoc* test were used to show the differences among the groups. Mann-Whitney test was applied for comparing the score of pathology between each experimental group and the sham groups. $P < 0.05$ was considered statistically significant.

RESULTS

Changes in kidney weight

Kidney weight/100 g of bodyweight (KW/100g BW) in all groups is shown in Figure 1. This ratio did not change in female CP treated animals compared with the female sham group, but there are significant differences between male CP treated and male sham groups ($P < 0.05$). However, treatment with SMT

alone increased the ratio in female animals unlike the male group. The results also indicated that co-administration of CP and SMT increased KW/100 g BW when compared with the CP alone treated group in females ($P < 0.05$). This was not observed in males.

Changes in serum Cr and BUN

Administration of CP significantly increased serum Cr and BUN levels in both male and female animals ($P < 0.05$). This is while co-administration of SMT and CP decreased the serum level of BUN in males significantly ($P < 0.05$) and in females insignificantly [Figure 1].

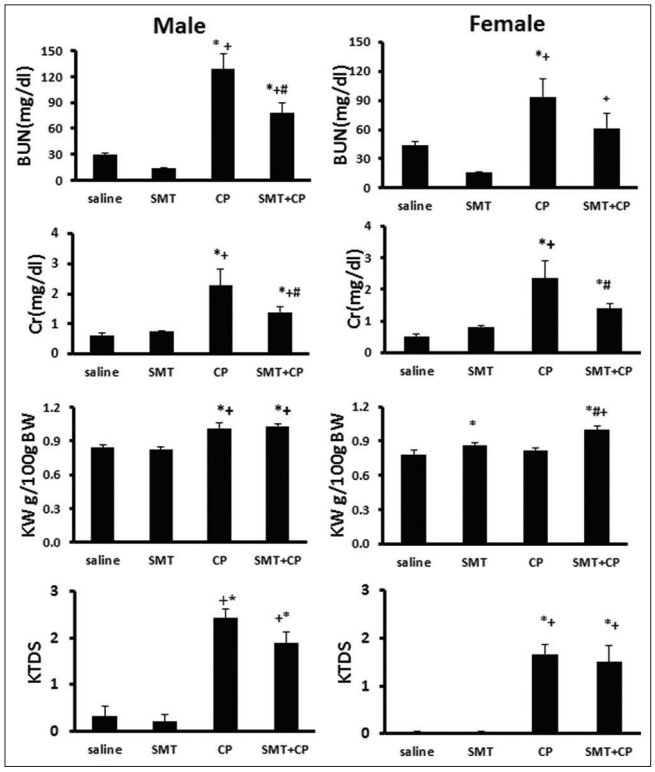

Figure 1: Compression of serum levels of BUN and Cr, and Kidney weight/100 g of bodyweight (KW g/100 g BW) and kidney tissue damage score (KTDS) in male and female rats treated with saline, CP, SMT, and combination of SMT and CP (SMT+CP) (data are expressed as mean ± SEM). The symbols indicates significant difference from (*) saline, (+) SMT, or (#) CP ($P < 0.05$)

Changes in serum MDA and nitrite levels

Change in serum level of MDA was measured in all groups [Table 1]. SMT alone increased the serum level of MDA significantly in females ($P < 0.05$) but not in males. However, co-administration of CP and SMT decreased the serum level of MDA in male, and increased in female ($P < 0.05$).

SMT, CP, and combination of both significantly decreased the serum level of nitrite in female ($P < 0.05$). However, a different result was obtained in male. CP alone increased the serum level of nitrite significantly ($P < 0.05$), while SMT or SMT plus CP decreased the serum level of nitrite [Table 1].

Changes in kidney histopathology

The CP-induced kidney damage was evaluated and scored by a pathologist. Significantly higher kidney damage scores were obtained in the CP-treated groups when compared with the sham group in both males and females ($P < 0.05$). Although therapy with SMT alone did not have any deleterious effects on kidney tubular structures, its combination with CP decreased kidney tissue damage score (KTDS) insignificantly in male. The images of kidney tissue in all groups are illustrated in Figure 2.

DISCUSSION

Our findings in the present study showed that inhibition of iNOS by SMT in CP treated model may decrease kidney toxicity in male. However, based on our data (KW, BUN, and KTDS), it seems that SMT therapy showed to be a more effective nephroprotectant agent against CP in male animals than female.

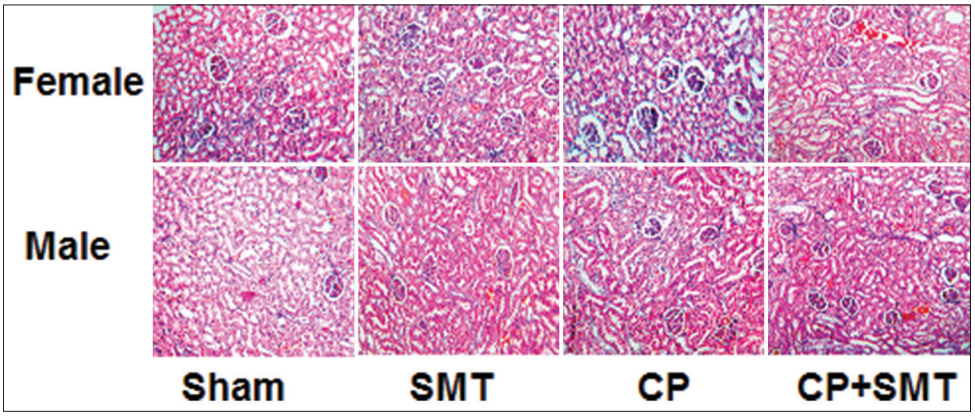

Figure 2: The images of kidney tissue in male and female rats treated with saline, CP, SMT, and combination of SMT and CP (SMT+CP)

Table 1: Serum and kidney levels of MDA and nitrite in male and female animals of the saline, CP, SMT, and SMT+CP groups (data are expressed as mean±SEM)

Gender	Male		Female	
Female group	Nitrite (nmole/L)	MDA (μmole/L)	Nitrite (μmole/L)	MDA (μmole/L)
Sham	19.06±3.66	16.75±2.35	20.78±3.98	1.3±0.26
SMT	12.36±1.94	14.2±2.81	10.16±1.90*	12.49±0.35*
CP	26.71±4.96*†	18±4.03	14.64±3.43*	2.49±0.35*†
SMT+CP	9.97±1.90*†	10.8±1.58*#	11.52±2.69*	15.56±2.04*#

Significant difference (P<0.05) from (*) sham, (†) SMT, or (#) CP.
MDA: Malondialdehyde, CP: Cisplatin, SMT: S-methylisothiourea hemisulfate, SEM: Standard error mean

NO plays a major role as a messenger molecule in most human organ systems including kidney. Physiologic concentrations of NO plays a role in the kidney function as a tonic vasodilator.[17] However, higher concentrations can be toxic, damaging cellular constituents such as DNA and inducing hypotension.[18] Transcriptional regulation of iNOS can be markedly induced, particularly by inflammatory cytokines, resulting in extremely large amounts of NO.[18]

In our previous study,[12] we showed that l-arginine may be protective against CP-induced nephrotoxicity in male, but it promotes the induced damage in female. Some researchers have demonstrated that selective iNOS inhibition reduces CP-induced nephrotoxicity and nitrosative stress in male, and they strongly suggested that in the experimental model, the NO production is toxic and iNOS is the main source of NO.[19] It has been documented that vitamin E plays a role in decreasing CP-induced nephrotoxicity via decreasing production of NO metabolites,[7] and NO production is gender-related.[13,14] In the present study, we used a selective iNOS inhibitor in both male and female to protect the kidney against CP-induced nephrotoxicity. However, our results showed that there is no significant difference between serum NO metabolite level in male and female groups. CP therapy could increase serum nitrite level in the male group but not in the female group. This finding is probably related to NO production by male sexual hormones.[20]

However, it is reported before that sex hormone estrogen could not protect the kidney form CP induced nephrotoxicity,[21,22] but the KW and KTDS in male rat treated with CP were significantly higher than in female animals.[9] In addition the serum level of MDA also was found to be lower in normal female that male,[23] and also in female rats treated with CP than male rat.[9] Therefore, it seems that the sex differences in this study may be related to many other parameters other than sex hormones.

In the present study, we showed that SMT therapy could decrease serum BUN and Cr levels in the male group. In this regard, some researchers believe that inhibition of iNOS production can increase plasma testosterone level and testosterone in turn can reduce plasma BUN and Cr levels.[24] According to the histological changes in kidneys, it seems that SMT therapy has a protective effect against CP-induced nephrotoxicity in the male group but not in the female group. So, considering the results obtained in the present study and our previous study,[12] we possibly can conclude that increase in NO production by l-arginine or decrease in NO production by SMT has beneficial effects on reduction of CP-induced nephrotoxicity in male. However, changes in NO level do not have any beneficial effect on reducing kidney damage induced by CP in female.

ACKNOWLEDGMENTS

This research was supported by Hormozgan University of Medical Sciences.

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Source of Support: This research was supported by Hormozgan University of Medical Sciences, **Conflict of Interest:** None declared.