A Combination of Vitamin C and Losartan for Cisplatin-induced Nephrotoxicity in Rats

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Introduction. The nephroprotective effect of co-administration of vitamin C and losartan as prophylaxis against cisplatin-induced nephrotoxicity (CIN) was evaluated.
Materials and Methods. Co-administration of vitamin C and losartan was compared with losartan (10 mg/kg), vitamin C (250 mg/kg), and placebo in 4 groups of rats with CIN. The prophylactic agents were injected daily for a period of 4 days, and on day 3, a single dose (6 mg/kg) of cisplatin was administrated. The animals were sacrificed 7 days later for pathological examination of the kidneys.
Results. Cisplatin prevented the animals' weight gain. The serum levels of creatinine and blood urea nitrogen increased within the groups with CIN, but no significant difference was observed between the groups. The prophylaxis has no effect on serum osmolality, total protein, or nitrite concentrations. The kidney tissue damage was scored, and losartan provided a lower damage score than vitamin C and a combination of vitamin C and losartan.
Conclusions. We concluded that co-administration of vitamin C and losartan was not more effective than the administration of vitamin C or losartan alone.

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Original Paper

INTRODUCTION

Widely used in chemotherapy of solid tumors, cisplatin imposes the risk of nephrotoxicity to the patient, as one of its major side effects of cisplatin.[1] In recent years, supplementations of different antioxidants known as nephroprotective agent have been studied.[2-4] Vitamin E and C, the most common antioxidants, have been investigated in different experimental models to demonstrate their effective role in prevention of cisplatin-induced nephrotoxicity (CIN).[2,3,5,6]

The angiotensin II receptor 1 blocker losartan is also an antioxidant, and it affects renal blood flow through blockade of vasoconstrictive action of angiotensin II.[7,8] Losartan was subjected to reduce the adverse effect of cisplatin in the renal system. However, Deegan and colleagues administrated low and high single doses of losartan (10 and 30 mg/kg) 2 hours prior to cisplatin injection, and they reported that treatment by losartan did not prevent development of CIN.[9] On the other hand, Saleh and colleagues used a single dose of losartan (60 mg/kg) and documented the protective role of losartan against CIN.[10] However, in both mentioned studies, chronic blockade of angiotensin II receptor 1 was nephroprotective against CIN.[9,10]

We previously demonstrated that prophylactic supplementation of vitamin E is more effective than co-administration of vitamin E and losartan or losartan alone to protect the kidney against CIN.[11] Since vitamin C is a more effective antioxidant than vitamin E to protect against CIN,[3] we designed a study to test the hypothesis of co-administration of losartan and vitamin C being effective in prevention of CIN.

MATERIALS AND METHODS

Adult male Wistar rats with a mean of 200.2 ± 2.57 g (Animal Centre, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this research project. The animals had free access to water and rat chow. This experimental procedure was approved in advance by Isfahan University Medical Sciences Ethics Committee.

Four groups of animals, 5 in each group, were randomly assigned as experimental groups. Rats in 4 of these groups received vitamin C, losartan, combined losartan and vitamin C, or normal saline (as placebo) before injection of cisplatin. Blood samples were obtained, and the rats were intraperitoneally injected daily with 10 mg/kg of losartan (group 1), 250 mg/kg of vitamin C (group 2), vitamin C and losartan in the same dosages (group 3), and normal saline (group 4; positive control) for 4 days. On day 3, rats in all the groups also were treated with 6 mg/kg of intraperitoneal cisplatin. Therefore, the animals in this protocol received prophylactic treatment or placebo 2 day before cisplatin injection, at the day of cisplatin injection, and 1 day after that. In order to obtain the normal kidney tissue and normal weight gain of the rats, 5 extra animals were selected to receive saline only during the study as a negative control group (group 5). Cisplatin (cis-Diammineplatinum (II) dichloride, code P4394), vitamin C (L-ascorbic acid, code A5960) were manufactured by Sigma (Sigma-Aldrich, Munich, Germany) and losartan (losartan potassium, code 61188) by Fluka (Sigma-Aldrich, Munich, Germany).

Seven days after cisplatin injection, blood and urine samples were obtained, and the rats were sacrificed. The kidney was removed rapidly for histopathological study. Serum was collected and stored in -20°C until measurement. The levels of serum creatinine, blood urea nitrogen (BUN), and total protein were determined using quantitative diagnostic kits (Pars Azmoon, Tehran, Iran). The osmolality of serum and urine were measured with vapour pressure osmometer Model 5500 (Wescor Inc, Logan, UT, USA). The serum and urine levels of nitrite (stable nitric oxide metabolite) were measured using a colorimetric assay kit (Promega Corporation, Madison, WI, USA) that involves the Griess reaction.

The kidney damage induced by cisplatin was scored blindly by two independent pathologists unaware of animal groups. The removed kidney was fixed in 10% neutral formalin solution and embedded in paraffin for histopathological staining. The staining was applied through periodic acid-Shiff staining to examine the tubular damage by 2 independent pathologists. Presence of tubular atrophy, cast, debris, and necrotic material in the tubular lumen and lymphocytes in interstitial tissue were considered. Based on the percentage of intensity of tubular lesions (ITL) as mentioned above, the tissues damages were scored as follows: ITL, 0 to 5%: score, 0; ITL, 6% to 20%: score, 1; ITL, 21% to 40%: score, 2; ITL, 41% to 60%: score, 3; ITL, 61% to 80%: score, 4; and ITL, > 80%: score, 5.

Data were expressed as mean ± standard error of mean. The paired *t* test was applied to compare the parameters within the groups. The 1-way analysis of variance was used to evaluate the effect of each treatment between the groups. To compare the scores given by 2 pathologists, the Spearman correlation was applied. To compare the score of pathology between the each experimental groups and the negative control group, the Mann-Whitney test was applied. Values of $P < .05$ were considered significant.

RESULTS

No weight gain was observed in cisplatin-treated groups, while the animals in the negative control group gained weight significantly by a mean value of 28 g ($P < .05$). Therefore, cisplatin prevented the normal process of weight gain, and vitamin C, losartan, or a combination of both as prophylaxis did not affect this process (Figure 1).

The BUN and serum creatinine levels were increased in all cisplatin-treated animals by 40% to 86% and 46% to 77%, respectively (Figure 2). In addition, no significant differences were observed between the groups.

The mean levels ranged from 5.4×10^9/L to 9.2

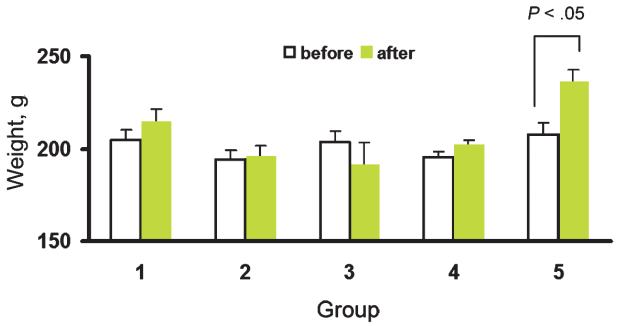

Figure 1. Body weight in experimental rat groups. Group 1 received losartan; group 2, vitamin C; group 3, vitamin C and losartan; and group 4, normal saline.

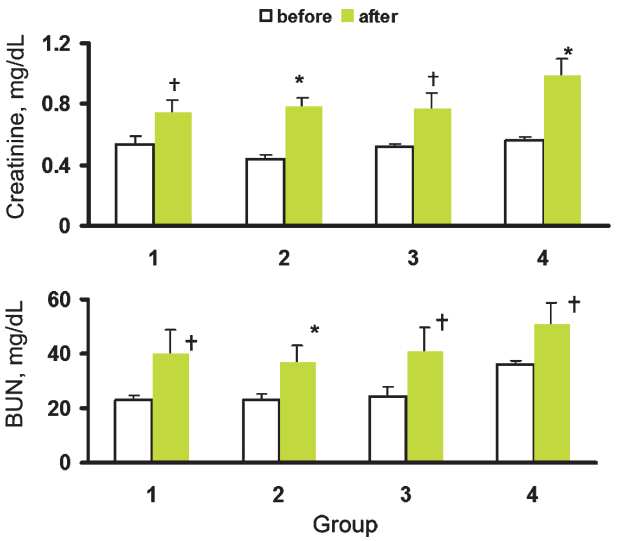

Figure 2. Blood urea nitrogen (BUN) and serum creatinine levels in experimental rat groups. Group 1 received losartan; group 2, vitamin C; group 3, vitamin C and losartan; and group 4, normal saline.
*P < .05 for before-after comparisons.
†P < .1 for before-after comparisons.

× 10^9/L for leukocyte count, 5.6 × 10^{12}/L to 6.3 × 10^{12}/L for erythrocyte count, 10.6 g/dL to 11.7 g/dL for hemoglobin, 29.5% to 32.4% for hematocrit, 763 × 10^9/L to 854 × 10^9/L for platelet count, and 14.4% to 26.2% for neutrophil count in cisplatin-treated rats. No significant differences were detected between the groups.

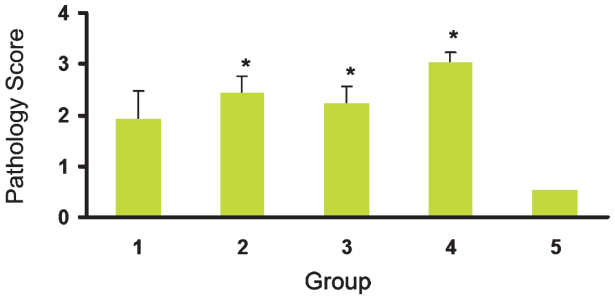

Figure 3. Kidney tissue damage score in experimental rat groups. Group 1 received losartan; group 2, vitamin C; group 3, vitamin C and losartan; and group 4, normal saline.
*P < .05 compared to the normal animals (group 5).

The final urine osmolality, nitrite level at the end of the experiment, and differences between the initial and final levels of serum total protein, osmolality, and nitrite are shown in the Table. No significant differences were observed between the experimental groups.

The kidney tissue from group 5 (negative control group) was considered as normal. The correlation between scores given by the two pathologists was compared and a significant correlation was obtained between the two observations (P < .001). The average score obtained for each animal and then for each group were considered as final damage tissue score. This data are demonstrated in Figure 3. More kidney damage was detected in groups 2, 3, and 4 when compared with group 5 (P < .05). The first group (losartan receivers as prophylaxis) indicates the lower score of pathology, and it was not significantly different from the negative control group (Figure 3). The images of tubular damage are demonstrated in Figure 4.

DISCUSSION

The main objective of this study was to investigate the prophylactic role of vitamin C and losartan

Serum and Urine Parameters in Cisplatin-treated Rats

	Experimental Groups*				
Parameter	**1**	**2**	**3**	**4**	***P***
Final urine osmolality, mosmol/kg	629.2 ± 254.8	541.2 ± 29.2	559.0 ± 174.2	485.2 ± 36.3	.94
Final urine nitrite, μmol/L	5.08 ± 0.55	6.71 ± 0.84	4.94 ± 0.68	5.82 ± 1.68	.62
Δ serum total protein g/dL	0.44 ± 0.60	1.18 ± 0.54	1.08 ± 0.48	1.34 ± 1.02	.81
Δ serum osmolality, mosmol/kg	-30.2 ± 20.0	-49.0 ± 26.4	-19.8 ± 18.7	-26.4 ± 84.3	.74
Δ serum nitrite, μmol/L	1.46 ± 1.58	0.83 ± 0.62	1.87 ± 0.77	-1.82 ± 2.10	.28

*Group 1 received losartan; group 2, vitamin C; group 3, vitamin C and losartan; and group 4, normal saline.

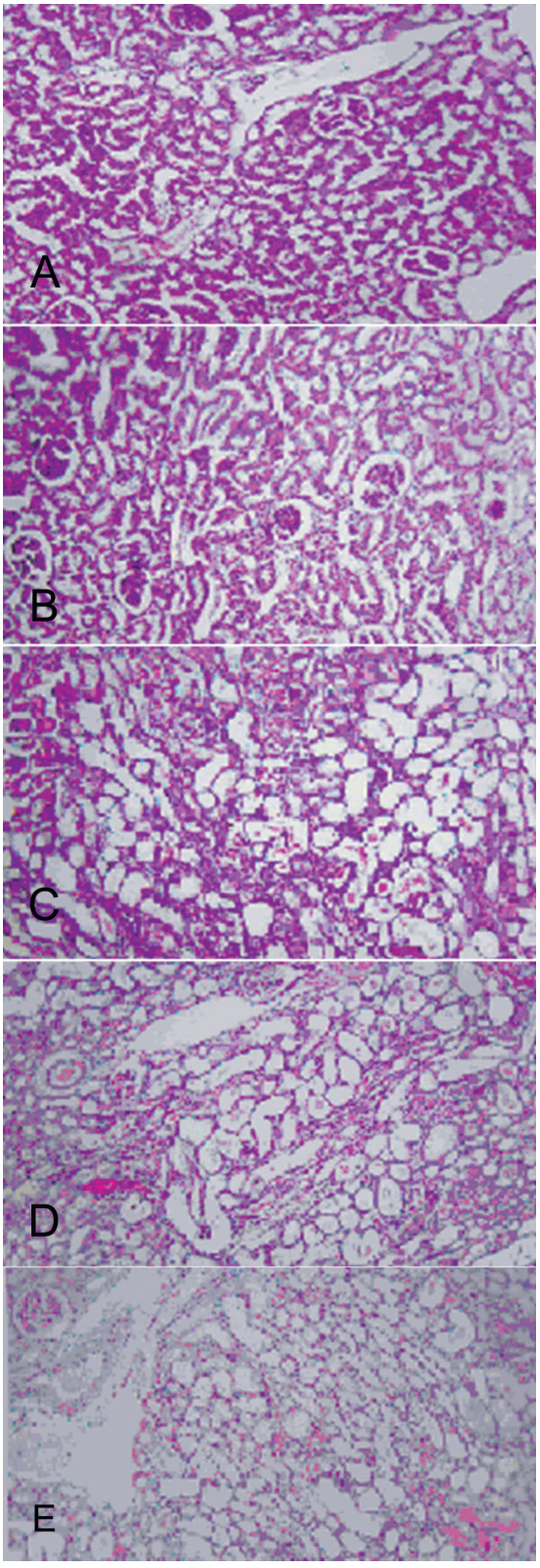

Figure 4. Tubular damage images in the experimental groups. **A**, Score zero in normal tissue. **B** to **E**, Scores 1 to 4, respectively, which correspond to up to 20%, 40%, 60%, and 80% presence of tubular atrophy, cast, debris, and necrotic material in the tubular lumen and lymphocytes in interstitial tissue.

co-administration in CIN. The major finding was related to the prophylactic effect of losartan (not a combination of losartan and vitamin C or vitamin C alone) that reduces cisplatin-induced kidney tissue damage. Serum creatinine and BUN levels and pathological damage scoring confirmed the existence of nephrotoxicity in this model.

Various reports are available showing that cisplatin affects weight, serum creatinine, BUN, serum protein, osmolality (or sodium), and nitric oxide levels.[3,5,6,9,10,12] On the other hand, some antioxidant substances or angiotensin II receptors blockers were administrated as nephroprotective agents against CIN.[2,3,5,6,9,10,13-15] Our results demonstrated that vitamin C, losartan, or a combination of both as prophylaxis reduced the levels of BUN and serum creatinine in cisplatin-treated animals, but the reduction was not significant (Figure 2). Antunes and colleagues reported that pretreatment with different doses of vitamin C before (10 minutes) cisplatin administration protected the kidney against the induced nephrotoxicity, and the protective effect of vitamin C was dose dependent.[5] In Deegan and colleagues' study, pretreatment with a single dose of losartan before (2 hours) cisplatin administration had no effect on BUN and serum creatinine levels.[9] In our model, the duration of pretreatment was 4 days, and therefore, reduction of BUN and serum creatinine levels, even nonsignificant, was expected; however, no clinical advantage for co-administration of vitamin C and losartan could be proposed.

Indeed, the pathological data indicated that the tissue damage in the first group (losartan receivers) decreased, and it had no statistical difference from the negative control group. This finding is interesting since currently there is no explanation why losartan alone was a better nephroprotective substance than a combination of losartan and vitamin C. Possibly, the effects of losartan on renal circulation is the main reason, and combination of vitamin C and losartan may provide an unknown drug interaction which limits the losartan effect.

There were some limitations in this study. We used single doses of cisplatin. Different results may be obtained with other or divided doses. The larger sample size may provide more significant results for measured parameters.

CONCLUSIONS

Our data indicated that the prophylactic administration of losartan was effective to protect against renal damage in cisplatin-treated rats, and losartan was the better nephroprotective substance than vitamin C and a combination of losartan and vitamin C in CIN; however, further studies are needed to find the significance of our findings.

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CONFLICT OF INTEREST

None declared.

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