

The Role of Gamma Amino Butyric Acid in Cisplatin-induced Nephrotoxicity in Streptozotocin-induced Diabetic Rats

Abstract

Background: Diabetes mellitus can change the risk of developing cancer. Cisplatin (CP) is a common antineoplastic drug. The major side effect of CP is nephrotoxicity. Gamma amino butyric acid (GABA) is an antioxidant agent that may have a protective role against CP-induced nephrotoxicity. The aim of the present study was to investigate the role of GABA in CP-induced nephrotoxicity in hyperglycemic male and female rats. **Materials and Methods:** Sixty male and female Wistar diabetic rats were used in ten experimental groups. GABA alone groups received GABA (50 $\mu\text{mol/kg/d}$ i.p.) for 12 days. CP alone groups received CP (2.5 mg/kg/d i.p.) for 6 days. Other groups received GABA in the form of therapy (T) + CP, prophylaxis (P) + CP, and prophylaxis-treatment (PT) + CP. Finally, blood samples were obtained, and animals were killed for kidney tissue investigation. **Results:** In female rats, the serum levels of creatinine (Cr) did not change by GABA rather than CP and also there were no significant changes in blood urea nitrogen to creatinine ratio (BUN/Cr). In male rats, plasma Cr level increased by GABA (P) and (T). Body weight loss was significantly different among groups ($P < 0.05$). BUN/Cr ratio significantly increased in CP and GABA (PT) + CP groups. In two genders, plasma Cr level significantly decreased in CP groups ($P < 0.05$). The kidney levels of malondialdehyde enhanced significantly in CP groups. **Conclusion:** Hyperglycemia has protective effect against CP-induced nephrotoxicity. GABA did not change this effect in female, but in male in the form of PT, GABA maintained it.

Keywords: Cisplatin, diabetes, gamma aminobutyric acid, gender, nephrotoxicity

Introduction

Diabetes and cancer are the most frequent illnesses in the world.^[1] It is well known that diabetes mellitus can change the risk of developing cancer, and there is some kinds of association among diabetes and pancreatic, non-Hodgkin's lymphoma, colorectal, prostate, endometrial, liver, breast, and renal cell cancers.^[2] Cisplatin (CP) as an important antineoplastic drug, is widely used in the therapy of solid tumors including head and neck, lung, testis, ovary, and breast tumors.^[3] Unfortunately, it has some side effects such as ototoxicity, gastrototoxicity, myelosuppression, and allergic reactions while the major side effect of CP is nephrotoxicity, which happens in about 30% of patients due to oxidative stress, apoptosis, or an inflammatory response.^[4]

Studies on some antioxidants such as Vitamins C and E, melatonin, and selenium demonstrated their useful role in the prevention of CP-induced

nephrotoxicity.^[5-8] On the other hand, studies have been indicated that uncontrolled streptozotocin (STZ)-induced diabetes in rats could protect renal tissue against damage induced by CP.^[9,10] Gamma amino butyric acid (GABA) (a known important neurotransmitter), presents in some inhibitory pathways in the central nervous system, but it is also found in nonneural tissues such as kidney and pancreatic islets.^[11-13] GABA also is an antioxidant that reduces oxidative stress induced by nephrectomy through increasing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and also decreasing lipid peroxidation.^[14] It is reported that GABA has protective effects against vasoconstriction and ischemia in the kidney,^[15,16] and it also has a renoprotective action on glycerol-induced acute renal failure.^[11] GABA has vasorelaxant effects^[17] and it can ameliorate CP nephrotoxicity in rats.^[18] GABA exerts antidiabetic effects by acting β -cell restoration and immune suppression^[19] and it could adjust insulin

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and glucagon secretion and could be used for type 1 diabetes therapy.^[20] In the current study, we investigated the role of GABA in CP-induced nephrotoxicity in STZ-induced diabetic rat model. On the other hand, it showed that CP-induced nephrotoxicity is sex-related,^[21] and the protective effects of some supplements are different in the two genders.^[22,23] Therefore, our study was carried out in both male and female diabetic rats.

Materials and Methods

Animals

Adult male (189 ± 3.47 g) and female (162 ± 3.02 g) Wistar rats (Animal Centre, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this study. Animals were housed at a room temperature of 23–25°C and 12 h light/12 h dark cycle with free access to water and rat chow. The experimental procedures were approved by the Isfahan University of Medical Sciences Ethics Committee.

Experimental protocol

Animals received a single dose of STZ (60 mg/kg i.p.). Five days later, blood glucose level was measured using glucometer (ACCU-CHEK Active, GC model, Germany) and the rates with blood glucose levels above 250 mg/dl were considered as diabetic rat. Then, 30 male and 30 female diabetic rats were divided into 10 groups (6 rats per each group). Female and male groups were assigned as groups 1–5 and groups 6–10, respectively.

Group 1 or 6 (named GABA): Diabetic rats received GABA (50 μ mol/kg/day i.p.) for 12 days.

Group 2 or 7 (named CP): Diabetic rats received CP (2.5 mg/kg/day i.p.) from the 7th day until 12th day.

Group 3 or 8 named GA (prophylaxis-treatment [PT] + CP): Diabetic rats received GABA (50 μ mol/kg/d i.p.) for 12 days, but from the 7th day, the animals were also treated with CP (2.5 mg/kg/d i.p.) until the end of experiment, 12th day.

Group 4 or 9 named GA (T + CP): Diabetic rats treated with GABA (50 μ mol/kg/d i.p.) and CP (2.5 mg/kg/d i.p.) simultaneously for 6 days.

Group 5 or 10 named GA (P + CP): Diabetic rats treated with GABA (50 μ mol/kg/day i.p.) for 6 days as prophylaxis. Then, the GABA was stopped and the animals received CP (2.5 mg/kg/d i.p.) for the next 6 days [Table 1]. The animals were weighed daily, and at the end of the study, blood samples were obtained and then the animals were sacrificed. Left and right kidneys were removed and weighted immediately for histopathologic investigation and measurements. The uterus was also removed and weighed (UW).

Measurements

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were determined using diagnostic

Table 1: Groups and the protocol of study

Gender	Group		Period of treatment and administration	
	Number	Name	1-6 days	7-12 days
Female	1	GABA	GABA	GABA
	2	CP	Saline	CP
	3	GA (PT) + CP	GABA	GABA + CP
	4	GA (T) + CP	Saline	GABA + CP
	5	GA (P) + CP	GABA	CP
Male	6	GABA	GABA	GABA
	7	Cp	Saline	CP
	8	GA (PT) + CP	GABA	GABA + CP
	9	GA (T) + CP	Saline	GABA + CP
	10	GA (P) + CP	GABA	CP

GABA: Gamma amino butyric acid, CP: Cisplatin, PT: Prophylaxis-treatment, GA: Gamma amino, T: Therapy, P: Prophylaxis

kits (Pars Azmoon Co., Tehran, Iran) and Autoanalyzer device (Technicon RA 1000, Ireland). The serum and urine levels of nitrite were measured using assay kit (Promega Corporation, Madison, WI, USA). The renal and serum levels of malondialdehyde (MDA) were measured by manual method using trichloroacetic acid and thiobarbituric acid.

Histopathological procedures

The left kidney was fixed in 10% neutral formalin solution and embedded in paraffin for hematoxylin and eosin staining to examine the tubular damage. A pathologist who was completely unaware of the study protocol and administered medications evaluated the damage. Kidney tissue damage score (KTDS) was graded from I to IV, based on the intensity of tubular lesions (hyaline cast, debris, vacuolization, flattening and degeneration of tubular cells, and dilatation of tubular lumen), whereas zero was assigned to normal tubules without any damage.

Statistical analysis

Data were expressed as mean \pm standard error of mean. Comparison of the groups by the body weight (BW) loss, kidney weight (KW), and levels of BUN, Cr, MDA, UW, and nitrite was performed by one-way ANOVA followed by the least significant difference test for multiple comparisons of significance. The histopathologic damage score of the groups was compared by the Kruskal–Wallis and Mann–Whitney U-tests. $P < 0.05$ was considered significant.

Results

Effect of cisplatin and gamma amino butyric acid on serum levels of creatinine, blood urea nitrogen, and blood urea nitrogen/creatinine ratio

In female diabetic rats, serum level of Cr increased significantly in the group of GABA when compared with other groups [$P < 0.05$, Figure 1]. In male diabetic rats,

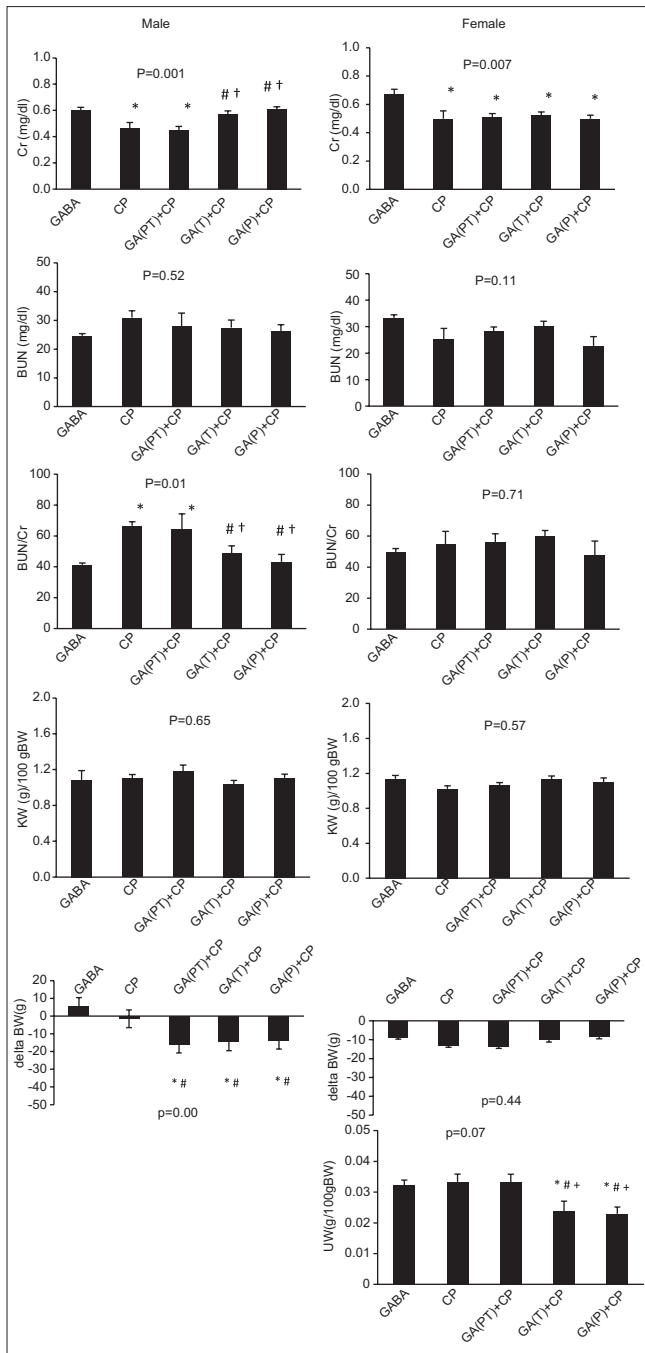


Figure 1: Serum levels of creatinine, blood urea nitrogen, and blood urea nitrogen/creatinine ratio and kidney weight per 100 g of body weight body weight change (delta body weight), uterus weight per 100 g of body weight, in 5 experimental groups in male and female streptozotocin-induced diabetic rats. Gamma amino butyric acid: Group received gamma amino butyric acid alone. Cisplatin: Group received cisplatin alone. Gamma amino (Prophylaxis-treatment) + cisplatin: Group received gamma amino butyric acid as prophylaxis and then treated with both gamma amino butyric acid and cisplatin. Gamma amino (therapy) + cisplatin: Group treated with gamma amino butyric acid and cisplatin simultaneously. Gamma amino (prophylaxis) + cisplatin: Group received gamma amino butyric acid as prophylaxis and then treated with cisplatin alone. *, # and † means significant difference from gamma amino butyric acid, cisplatin and gamma amino (prophylaxis-treatment) + cisplatin groups, respectively, $P < 0.05$

different results were seen. The serum level of Cr decreased significantly in the groups of CP and GA (PT) + CP when

compared with GABA alone group ($P < 0.05$). In addition, in male diabetic rats, serum level of Cr in groups GA (P) + CP and GA (T) + CP increased significantly in comparison with the CP alone and GA (PT) + CP groups ($P < 0.05$, Figure 1). However, no significant difference was detected statistically in the serum level of BUN between the groups neither in male ($P < 0.52$) nor in female ($P < 0.11$).

The data related to BUN/Cr ratio did not indicate any difference in the female groups. However, in male groups, the serum level of BUN/Cr ratio was higher in CP and GA (PT) + CP groups, which was significantly different from GABA group ($P < 0.05$, Figure 1).

The effect of cisplatin and gamma amino butyric acid on body weight, kidney weight, uterus weight, and kidney tissue damage score

In general, BW decreased in all the female groups with no significant difference between the groups. However, in male diabetic rats, the significant weight reduction was observed in all the three treated groups with GABA and CP when compared with GABA or CP alone treated groups ($P < 0.05$). No significant difference in KW was observed between the groups neither in male nor in female rats [Figure 1]. However, UW in the groups of GA (T) + CP and GA (P) +CP was reduced significantly when compared with other groups [$P < 0.05$, Figure 1]. The images of kidney tissues are shown in Figure 2 and kidney tissue damage score is illustrated in Table 2.

The effect of cisplatin and gamma amino butyric acid on serum and kidney levels of nitrite and malondialdehyde

No significant changes were observed in serum and kidney levels of nitrite in two genders [Table 3], but in male groups, the serum level of MDA in GABA alone treated group was significantly lower than others groups ($P < 0.05$). On the other hand, the kidney level of MDA in male and female groups received CP alone elevated significantly when compared with GABA alone treated groups [$P < 0.05$, Table 3].

Discussion

The principal purpose of this study was to investigate the protective effect of GABA on CP-induced nephrotoxicity in female and male diabetic rats. In normal rats, CP induced nephrotoxicity recognized by increased serum BUN and Cr level as well as KW.^[24,25] Our results showed that serum levels of BUN and Cr remained within the normal range in both female and male diabetic rats that received CP, and no change was observed in KW and KTDS. In fact, diabetes itself protects the kidney from CP side effects which was also accepted by others.^[9,10] It is reported that patients with polymorphisms in the gene OCT2 (transport regulator gene platinum in the kidney cells) exposed lower risk of renal toxicity^[4] while after 7 days, STZ-induced diabetes OCT2 expression decreased more than 50%.^[26] Furthermore, it

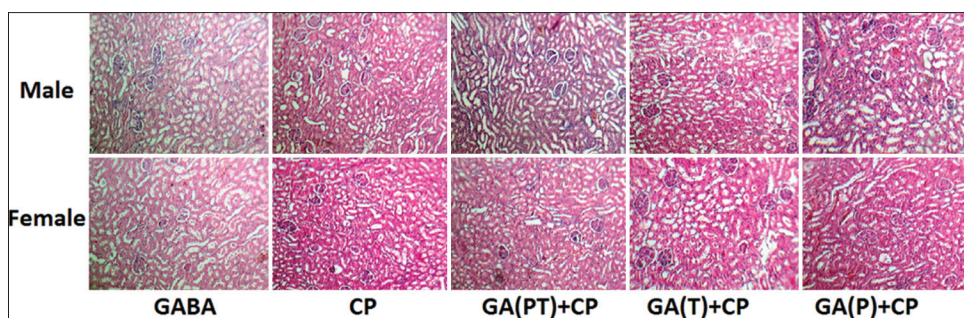


Figure 2: The images (magnification 100) of kidney tissue in 5 experimental groups in male and female streptozotocin-induced diabetic rats. Gamma amino butyric acid: Group received gamma amino butyric acid alone. Cisplatin: Group received cisplatin alone. Gamma amino (prophylaxis-treatment) + cisplatin: Group received gamma amino butyric acid as prophylaxis and then treated with both gamma amino butyric acid and cisplatin. Gamma amino (therapy) + cisplatin: Group treated with gamma amino butyric acid and cisplatin simultaneously. Gamma amino (prophylaxis) + cisplatin: Group received gamma amino butyric acid as prophylaxis and then treated with cisplatin alone

Table 2: The kidney tissue damage score in experimental groups

Gender	Group Treat	KTDS				n	
		0	1	2	3		4
Male	GABA		2	4			6
	CP		4	2			6
	GA (PT) + CP		3	3			6
	GA (T) + CP			6			6
	GA (P) + Cp			6			6
Female	GABA			3	3		6
	CP		4	2			6
	GA (PT) + CP	1	4	1			6
	GA (T) + CP		4	2			6
	GA (P) + Cp		5		1		6

Grading scale is as follows: 0=Normal, 1=Minimal damage (5-25%), 2=Mild damage (25-50%), 3=Moderate damage (50-75%), 4=Severe damage (>75%). KTDS: Kidney tissue damage score, GABA: Gamma amino butyric acid, CP: Cisplatin, PT: Prophylaxis-treatment, GA: Gamma amino, T: Therapy, P: Prophylaxis

showed that high blood glucose level has protective effect against CP-induced nephrotoxicity.^[27] Therefore, it seems that with increased urinary CP excretion^[27,28] and decrease of OCT2 expression in diabetic rat model^[26] as well as protective effects of high blood glucose, the serum level of Cr did not alter by CP similar to what Soltani *et al.* reported.^[9] Our results showed that in male or female diabetic rats, administration of GABA is not needed to attenuate CP-induced nephrotoxicity because diabetes itself controls this side effect. Kim *et al.* showed GABA effect on Cr clearance and prevents progression of renal failure.^[11] Our results for serum level of Cr were different between male and female. This difference may relate to sex hormone because estrogen has antioxidant properties.^[29] Vafapour *et al.* reported that GABA decreased the serum level of Cr in renal ischemic/reperfusion in female, but not in male rats.^[30] In clinical medicine, the causes of acute kidney injury are often divided into prerenal, intra renal, and postrenal. The BUN/Cr ratio is used to distinguish prerenal dysfunction,^[31] and it is also considered as a renal function indicator. The increase of this proportion

may indicate decline in renal perfusion and glomerular filtration rate.^[32] In our study, BUN/Cr increased in CP and GA (PT) + CP group presumably because CP reduced renal perfusion. CP administration declined BW in two genders. That may be owing to gastrointestinal disorders^[33] and may be STZ has been producing gastrointestinal lesion. On the other side, hyperglycemia and hypoinsulinemia cause decline in the BW,^[34-36] particularly in type 1 diabetes.^[37] In male diabetic rats, GABA alone could improve BW loss that may be due to the regulation of appetite by GABA which confirms other studies.^[38] CP administration leads to increased KW that is related to kidney tissue damage intensity.^[39] In the present study, as expected, KW does not change significantly between the groups presumably due to the protective effect of diabetes and GABA on CP-induced nephrotoxicity and histological data confirmed it. According to some studies, GABA improves increased KW induced by hyperglycemia in diabetic rats' model induced by STZ.^[40] There are different opinions about the effect of GABA on UW. Studies show that hyperglycemia in STZ-induced diabetic rats leads to endometrial and myometrium atrophy and loss UW.^[41] CP also decreases UW via creating apoptosis and necrosis in the tissue,^[42,43] In the present study, GABA alone does not change UW similar to what Takeshima *et al.* reported,^[44] while GABA in the form of prophylaxis and therapy, decreased UW, and this finding was in agreement with other documents.^[30] MDA, as the final product of lipid peroxidation, is one of the biomarkers of oxidative stress.^[45] CP administration is leading to lipid peroxidation and increase the MDA level.^[46] In the present study, CP alone increases the MDA level of kidney tissue in two genders. When compared with GABA alone, GABA reduces oxidative stress through increased activity of antioxidant enzymes and decreased lipid peroxidation;^[14,18] also, we observed that GABA decreased serum MDA level in male diabetic rats. We are wise that GABA is upper in the male than female.^[47] NO produced by endothelial NO synthase (eNOS) is a vascular relaxation factor.^[48] The vasodilator action of NO on vascular smooth muscle cells is well known.^[49] NO is unstable and rapidly oxidized to nitrite (NO₂).^[50,51] Hence, in this study, NO₂

Table 3: Levels of nitrite and malondialdehyde in serum and kidney in all experimental groups

Gender and groups		Serum nitrite	Kidney nitrite	Serum MDA	Kidney MDA
Gender	Group	($\mu\text{mol/L}$)	($\mu\text{mol/g tissue}$)	($\mu\text{mol/L}$)	(nmol/g tissue)
Male	GABA	10.91 \pm 1.63	0.14 \pm 0.22	4.62 \pm 0.15	6.32 \pm 0.80
	CP	8.20 \pm 0.93	0.16 \pm 0.12	8.62 \pm 1.20*	10.08 \pm 1.15*
	GA (PT) + CP	11.48 \pm 1.75	0.23 \pm 0.88	10.40 \pm 1.77*	8.38 \pm 0.82
	GA (T) + CP	9.93 \pm 1.42	0.22 \pm 0.26	9.52 \pm 1.48*	11.33 \pm 0.75*
	GA (P) + CP	10.41 \pm 2.25	0.17 \pm 0.13	9.65 \pm 0.95*	10.38 \pm 1.21*
	<i>P</i>	0.65	0.57	0.02	0.01
Female	GABA	17.43 \pm 2.13	0.22 \pm 0.32	7.70 \pm 0.98	6.84 \pm 1.03 [#]
	CP	16.17 \pm 5.66	0.20 \pm 0.42	8.27 \pm 0.80	11.06 \pm 1.31
	GA (PT) + CP	12.58 \pm 0.98	0.22 \pm 0.49	7.47 \pm 0.55	7.14 \pm 0.44 [#]
	GA (T) + CP	16.62 \pm 1.90	0.16 \pm 0.28	8.27 \pm 1.28	6.64 \pm 1.09 [#]
	GA (P) + CP	12.42 \pm 2.85	0.17 \pm 0.37	5.28 \pm 0.84 ^{#,&}	5.71 \pm 0.90 [#]
	<i>P</i>	0.68	0.79	0.15	0.08

*,#,&Significant difference from GABA, CP, and GA (T) + CP groups respectively $P < 0.05$. GABA: Groups received GABA alone, CP: Groups received cisplatin alone, GA (PT) + CP: Groups received GABA as prophylaxis and then treated with both GABA and CP, GA (T) + CP: Groups treated with GABA and CP simultaneously, GA (P) + CP: Groups received GABA as prophylaxis and then treated with CP alone. GABA: Gamma amino butyric acid, CP: Cisplatin, PT: Prophylaxis-treatment, GA: Gamma amino, T: Therapy, P: Prophylaxis, MDA: Malondialdehyde

was measured as an index for renal damage. Inhibition of eNOS leads to an increase in renal vascular resistance and decreased blood flow and glomerular filtration.^[52] On the other hand, according to some studies, inducible NO synthase increased after damage epithelial cells in response to the oxidative stress.^[53] CP damages the kidney tubules and lower production of endothelial NO.^[54]

Conclusion

Hyperglycemia has protective effect against CP-induced nephrotoxicity. GABA did not change this effect in female, but in male in the form of PT, GABA maintained it.

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Conflicts of interest

There are no conflicts of interest.

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