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Effect of Fructose-1,6-bisphosphate on the Nephrotoxicity Induced by Cisplatin in Rats

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Abstract—Cisplatin is one of the most active cytotoxic agents in the treatment of cancer, but its clinical use is frequently limited by nephrotoxicity. The study presented here attempted to evaluate the effect of fructose-1,6-bisphosphate in the cisplatin-induced nephrotoxicity in rats. The drugs were administered intraperitoneally as a single dose: sodium chloride 0.9%, cisplatin (6 mg/kg), fructose-1,6-bisphosphate (500 mg/kg), and cisplatin plus fructose-1,6-bisphosphate (6 and 500 mg/kg, respectively). The use of cisplatin resulted in significant elevation of serum creatinine and urea. The group that received cisplatin plus fructose-1,6-bisphosphate presented a significantly lower level of creatinine and urea compared to the cisplatin group. Acute tubular necrosis was demonstrated in the animals that received cisplatin and a less severe one in the cisplatin plus fructose-1,6-bisphosphate group. Fructose-1,6-bisphosphate has a protective effect over renal function and renal parenchyma in a rat experimental model of cisplatin-induced nephrotoxicity. The anti-inflammatory effect of fructose-1,6-bisphosphate confirms its protective effect in cases of cellular injury.

KEY WORDS: cisplatin; fructose-1,6-bisphosphate; nephrotoxicity; tubular necrosis.

INTRODUCTION

Cisplatin (*cis*-dichlorodiammineplatinum II) is a synthetic anticancer drug extensively clinically used in the treatment of several human malignancies [1]. In spite of that, its full clinical utility is limited by the induced renal toxicity [2]. Cisplatin nephrotoxicity is a frequent complication in the treatment with platinum compounds, and a major dose-limiting toxicity for this drug has been demonstrated in both adults and children [3].

Fructose-1,6-bisphosphate is an intermediate metabolite of the glycolytic route [4], and its therapeutic effects on kidneys, heart, liver, intestine, brain, and lung ischemic damage have been previously described [5, 6]; as well as a protective capacity in other pathologies, such as toxic shock and lesion [7]. Fructose-1,6-bisphosphate is capable of reducing carrageenan-induced edema in the rat paw [8], demonstrating its anti-inflammatory role. The protective effects presented by this sugar result from its ability to maintain the intracellular levels of ATP and calcium, avoiding cellular death due to energy deficit [9]. Markov et al. [10] reported a preservative effect in myocardial cells after fructose-1,6-bisphosphate exposure, as well as an increase in ATP levels and creatinophosphate. Oliveira et al. [11] reported that galactosamine-induced cell damage in hepatocytes was protected when fructose-1,6-bisphosphate was used, as well as lower apoptosis indexes were demonstrated in the sugar control group.

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Recent studies have focused on alternatives to reduce the cytotoxic impact of cisplatin, and several strategies have been explored to reduce the side effects of cisplatin therapy [1]. The frequent limitation in the clinical practice use of cisplatin due to its nephrotoxicity motivated us to evaluate nephroprotection mechanisms and drugs to attenuate this adverse effect. Thus, the study presented here attempted to evaluate the effect of fructose-1,6-bisphosphate in the cisplatin-induced nephrotoxicity in rats.

MATERIAL AND METHODS

Adult male Wistar rats (*Rattus norvegicus*) bred in our laboratory (weighing 200–300 g) were used. All of them had the same ancestry and socialization, as well as free access to food and water. The animals were maintained in accordance with the *Guiding Principles in the Care and Use of Animals* approved by the Council of the American Physiological Society. In all experiments, animals were divided into four groups and treated as follows: (1) group NaCl—sodium chloride 0.9%; (2) group DDP—cisplatin 6 mg/kg; (3) group FBP—fructose-1,6-bisphosphate 500 mg/kg; and (4) group DDP+FBP—cisplatin 6 mg/kg plus fructose-1,6-bisphosphate 500 mg/kg. The experiments were performed on four different days: 1, 4, 8, and 12 days after treatment.

All treatments were administered intraperitonially on day 0. The animals were killed after 1 (n=6), 4 (n=6), 8 (n=6), and 12 (n=6) days after treatment in each group. After that, blood samples were collected and the kidneys were removed. Serum creatinine and urea were measured through the Jaffé and urease methods, respectively. For the histological analyses, kidney tissues were stained with hematoxilin and eosin. A light-microscopical analysis was performed, and a scoring system was used: 0, normal renal architecture; 1, minimal necrosis of tubular cells; 2, moderate to severe tubular necrosis, no proteinic material in the tubules; and 3, severe tubular necrosis, proteinic material in tubules. All slides were evaluated by an investigator blinded to the experimental groups.

The results were statistically evaluated by analysis of variance with Duncan *post hoc* test using SPSS (Statistical Package for the Social Sciences) 12.0 software and were expressed as means ± SD. The level of statistical significance was defined as $P<0.05$.

RESULTS

Cisplatin induced a body weight loss that was more pronounced on day 8 (Table 1). The treatment with fructose-1,6-bisphosphate attenuated this reduction in the body weight leading to a quicker, but statistically not significant, recovery.

Tables 2 and 3 show the mean and standard deviation results of serum creatinine and urea in the different days. The results demonstrate that there was no variation in the creatinine and urea levels on day 1. When creatinine results were compared in the same day, between different groups, a statistically significant increase on day 4 was identified when the DDP group was compared to the others. In the same way, on day 8, a statistical difference between DDP and DDP + FBP compared to the others was shown. When urea levels were analyzed in the same day, between groups, statistical differences on days 4, 8, and 12 were demonstrated when DDP and DDP + FBP were compared between each other and to the other groups. When results were analyzed in the same groups, there was a significant increase in the creatinine levels for the DDP group when days 4 and 8 were compared to the other days. A significant difference in the urea levels was also identified for the DDP + FBP group when day 4 was compared to the others.

Histological analysis are shown in Table 4 and illustrated in Fig. 1a–c. There were no differences in the macroscopic analysis. Indeed, no alterations were observed in the microscopic analysis in both the NaCl and FBP groups. When analyzing day 1, 50% of the animals treated with cisplatin showed acute tubular necrosis grade 1, as well as animals treated with DDP + FBP, which presented 25% of acute tubular necrosis. On day 4, 75% of the animals in the DDP group showed score 2.0 alterations, and in the group treated with DDP + FBP, a lesion score of 1.0 was observed in 25% of the animals. When day 8 was analyzed, the maximum level of lesion (score 3.0) was observed in the DDP and DDP + FBP

Table 1. Evaluation of Body Weight (g) ± SD in the Different Groups and Days of Treatment

Group	Day 0	Day 1	Day 4	Day 8	Day 12
Nacl	236±6	238±10	242±8	249±10	248±12
DDP	286±12	285±20	258±28	231±42	243±30
FBP	265±8	267±16	288±26	288±18	305±12
DDP + FBP	286±11	286±12	264±24	280±22	283±20

Table 2. Evaluation of Serum Creatinine (mg/dL)±SD in the Different Groups and Days of Treatment

Group	Day 1	Day 4	Day 8	Day 12
Nacl	0.4±0.07	0.3±0.06	0.3±0.08	0.4±0.05
DDP	0.5±0.10	1.5±0.20$^{\#*}$	1.3±0.10$^{\#*}$	0.6±0.10
FBP	0.4±0.07	0.5±0.20	0.5±0.10	0.4±0.10
DDP + FBP	0.5±0.03	0.7±0.20	0.6±0.20$^{\#}$	0.6±0.40

* $P<0.05$ when different days were compared in the same group during the study; $^{\#}$ $P<0.05$ when different groups were compared in the same day

groups. In the DDP group, the alterations were observed in 100% of the animals, although in the DDP + FBP group, in only 50% of the animals. Finally, when analyzing the presence of alterations in the DDP group, only 25% of the animals presented score 3.0, whereas 50% showed a score of 1.0 and 25% a score of 2.0. In the DDP + FBP group, the alterations were observed in 75% of the animals, where 25% presented a score of 2.0 and 50% a score of 1.0.

DISCUSSION

One day after the cisplatin administration, the animals showed signs of the systemic toxicity of the drug, observed indirectly through the alteration in the body weight. The alterations were observed more severely in the DDP group when compared to the others. The weight loss was attenuated in the DDP + FBP group. The animals that received DDP presented symptoms often associated with the uremia syndrome (prostration, diarrhea, anorexia), which was less frequent in the DDP + FBP, indicating in a subjective way the attenuation of the cisplatin toxicity by the fructose-1,6-bisphosphate. The body weight alterations were already described in other studies with cisplatin, but none evaluated the protective effect of fructose-1, 6-bisphosphate in this effect.

Table 3. Evaluation of the Serum Urea (mg/dL) + SD in the Different Groups and Days of Treatment

Group	Day 1	Day 4	Day 8	Day 12
Nacl	36.2±3.6	38.7±3.0	37.1±3.7	35.5±2.5
DDP	53,5±7.5	108.0±39.6$^{\#}$	157.2±57.0$^{\#}$	72.7±14.3$^{\#}$
FBP	52.3±12.0	58.6±8.4	38.1±13.0	52.5±8.9
DDP + FBP	51,2±23.2	87.8±19.0$^{\#*}$	45.7±15.7$^{\#}$	70.9±34.6$^{\#}$

* $P<0.05$ when different days were compared in the same group during the study; $^{\#}$ $P<0.05$ when different groups were compared in the same day

Table 4. Histological Analysis (Mean of Scores) in the Different Groups and Days of Treatment

Group	Day 1	Day 4	Day 8	Day 12
Nacl	0	0	0	0
DDP	0.5	1.2$^{\#*}$	3.0$^{\#*}$	1.2$^{\#*}$
FBP	0	0	0	0
DDP+FBP	0.2	0.2	1.2$^{\#*}$	1.2$^{\#*}$

* $P<0.05$ when different days were compared in the same group during the study; $^{\#}$ $P<0.05$ when different groups were compared in the same day

Cisplatin has emerged as a main chemotherapeutic agent, although its clinical use may be limited by the potential nephrotoxicity [12]. Kidneys are dynamic organs and represent the major control system maintaining the body homeostasis, thus being affected by many chemicals and drugs. Excretion of cisplatin is predominantly renal. Cisplatin accumulates in the renal tubular cells approximately five times its extracellular concentration. Consequently, the kidney is considered to be the primary target organ for cisplatin toxicity. The impairment of the kidney function by cisplatin may occur either acutely or after repeated treatment. Therefore, it is recognized as the main side effect and the dose-limiting factor associated with its use [1]. The present study examined the protective effects of the fructose-1, 6-bisphosphate in the tubular segments function and morphology after treatment with the nephrotoxin cisplatin.

Cisplatin-induced nephrotoxicity results in severe nephropathy involving acute renal failure. Acute renal failure caused by cisplatin in rats exhibits histological alterations of the renal tubular cells. These changes are associated with the renal function loss, including severe reductions in glomerular filtration and creatinine clearance, and increased levels of serum creatinine [13]. Although the creatinine and urea do not represent the better way to evaluate the renal function, they are used in the clinical practice to this kind of evaluation. Acute renal failure is defined as the abrupt loss, in hours or days, of the renal function, sufficiently to cause the urea and creatinine accumulation. Our results demonstrated that the increase in the creatinine levels was higher in the days 4 and 8 after the administration of cisplatin, which is in accordance with previous published data that demonstrated this effect along with a urinary concentration alteration [12]. When the FBP-treated animals were analyzed, none of the days and groups developed acute renal failure. The results obtained with the laboratory analysis on the day 12 showed the partial recuperation in the creatinine and urea serum levels, as well as described in previous studies.

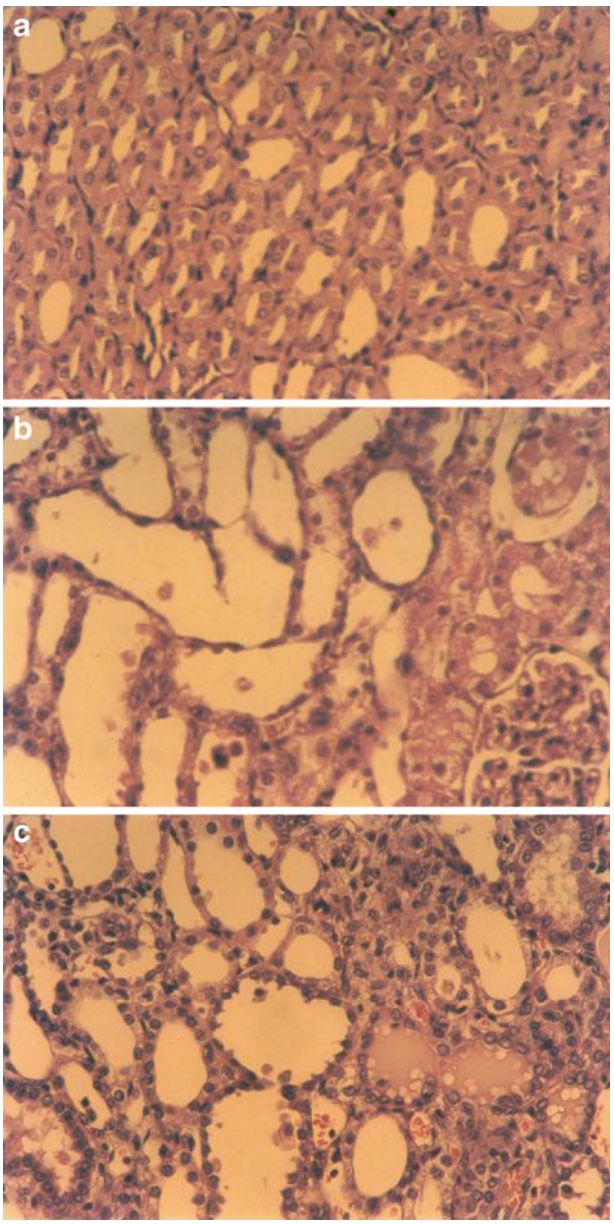

Fig. 1. Histological assessment of the kidney. **a** NaCl-treated animal as control. **b** Areas of severe tubules necrosis (score 3.0), day 8 after the DDP administration. **c** Renal tubular epithelium showing moderate ATN (score 2.0) in the DDP+FBP group on day 12.

Cisplatin causes tubular injury through multiple mechanisms, including hypoxia, the generation of free radicals, inflammation, and apoptosis [13]. Cisplatin is known to accumulate in the renal epithelial cells mitochondria and induce the formation of reactive oxygen species primarily by decreasing the activity of antioxidant enzymes by depleting intracellular concentrations of reduced glutathione and also by inducing the membrane lipid peroxidation [14].

Renal damage can be caused by the direct effect of some drugs in the polarity of the cellular membrane and in the distribution and function of the Na^+/K^+-ATPase. *In vitro* studies, performed in rat hepatocytes demonstrated, after galactosamine-induced toxicity, that in the presence of fructose-1,6-bisphosphate the Na^+/K^+-ATPase is not altered, supporting a potential role of fructose-1,6-bisphosphate in the cellular protection.

The kidney, in a situation of acute tubular necrosis induced by ischemia, demonstrates a cellular ATP level deficiency, as well as an alteration in the intracellular potassium and sodium levels. When the Na^+/K^+-ATPase activity is affected, an alteration in the electrolytes levels is observed. Previous data suggest that the FBP has the ability of inducing membrane transport alterations through the stabilization of the potassium channels, preventing the reduction of the intracellular levels and maintaining the ATP levels. The acute tubular necrosis is also associated with the alteration of the calcium intracellular levels, a potential way of injuring the organelles structures. Fructose-1,6-bisphosphate is a calcium chelator, leading, in its presence, to a cytoprotective effect.

Nitric oxide (NO) has been suggested to play an important role in the cisplatin-induced nephrotoxicity [13]. Cisplatin has been shown to cause hypoxic injuries along with reduced renal hemodynamics. An intrarenal vasoconstriction may be related to an imbalance between vasoconstrictors and vasodilators, in which NO play an important role. The expression of endothelial nitric oxide synthase (eNOS) is decreased, whereas the expression of inducible nitric oxide synthase (iNOS) is increased in cisplatin-induced acute kidney injury. The constitutive activity of eNOS preserves the renal function, and the induction of iNOS produces tissue injury. The decrease of eNOS may thus be causally related to a decrease of renal blood flow, whereas the increase of iNOS may be responsible for a cytotoxic injury. Moreover, the cisplatin-induced acute kidney injury has been in part related to an inflammatory response [12]. Cyclooxygenase 2 (COX-2) catalyzes the production of prostaglandins, which represents an important step in the inflammatory process [15]. Fructose-1,6-bisphosphate is capable of inducing a protective effect by attenuating the production of prostaglandin E and the expression of COX-2, as well as controlling the secretion of cytokines and the production of nitric oxide [16]. Thus, the potential COX-2 inhibitors have been considered as anti-inflammatory agents [15]. The anti-inflammatory

effect of fructose-1,6-bisphosphate confirms its protective effect in cases of cellular injury, for which it has been used experimentally in acute myocardial infarction and renal and cerebral ischemia based on its ability to inhibit the production of inflammatory mediators [16].

In the present study, the acute renal failure was clearly demonstrated after the administration of cisplatin, as well as the cytoprotective effect of FBP. The cellular protective effects of the fructose-1,6-bisphosphate were demonstrated in several previous studies, and present data show a clear demonstration of this effect in the renal system. The results presented here demonstrate that the nephroprotection of fructose-1,6-bisphosphate is observed through physiological analysis of creatinine and urea levels, as well as through histological parameters, evidenced by a decrease in the substances concentrations and in the acute tubular necrosis scores. The cytoprotection of FBP, already demonstrated in other organs, is also described here in the nephrotoxicity induced by the cisplatin.

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