Effect of glutamine on the total antioxidant system of rats subjected to renal ischemia and reperfusion[1]

Efeito da glutamina no sistema antioxidante total de ratos submetidos a isquemia e reperfusão renal

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ABSTRACT

PURPOSE: To evaluate the protective effects of glutamine administered before renal ischemia–reperfusion on plasma antioxidant protection, and lung and renal tissue injury.

METHODS: 33 rats underwent right nephrectomy. On the eighth postoperative day, animals were randomized into three groups (n=11): glutamine, control and sham. Each group of animals received, by gavage, a particular diet for 7 days. On day 14 following nephrectomy, the animals were subjected to left renal ischemia-reperfusion. After this, blood samples were collected and the animals were killed. At necropsy the kidney and lung were removed for histology.

RESULTS: The levels of total antioxidant capacity were higher in the glutamine group and control group compared with the sham group. The levels of glutathione peroxidase in both the sham and glutamine groups were higher when compared with the control group ($p<0.05$). The level of superoxide dismutase in the sham group was clearly higher than that in the glutamine and control groups. Histological examination showed no differences between the three groups.

CONCLUSION: Prior intake of glutamine improves total antioxidant capacity and increases glutathione peroxidase levels in rats subjected to renal ischemia–reperfusion.

Keywords: Glutamine. Ischemia. Renal Artery Obstruction. Kidney Transplantation. Free Radicals. Rats.

RESUMO

OBJETIVO: Avaliar os efeitos na proteção antioxidante plasmática e na lesão tecidual renal e pulmonar da glutamina oral administrada precedendo a isquemia/ reperfusão renal.

MÉTODOS: Trinta e três ratos foram submetidos à nefrectomia à direita. No oitavo dia de pós-operatório, os animais foram randomizados em três grupos (n=11): glutamina, controle e sham. Cada grupo de animal recebeu por gavagem uma dieta distinta por sete dias. Ao final do 14º dia da nefrectomia procedeu-se a isquemia renal esquerda e posterior reperfusão. A seguir procedeu-se a coleta de sangue, eutanásia e retirada do rim e pulmões para análise histológica.

RESULTADOS: Os níveis de capacidade antioxidante total foram maiores no grupo glutamina e grupo controle em relação ao grupo sham. Os níveis de glutationa peroxidase nos grupos sham e glutamina foram mais elevados quando comparados com o grupo controle ($p<0,05$). A dosagem de superóxido dismutase foi maior no grupo sham quando comparado com os grupos glutamina e controle. Não houve diferença na análise histológica do rim e pulmão entre os grupos.

CONCLUSÃO: O uso de glutamina antecedendo a isquemia reperfusão renal melhora os níveis da capacidade antioxidante total e eleva a glutationa peroxidase em ratos submetidos a isquemia-reperfusão renal.

Descritores: Glutamina. Isquemia. Obstrução da Artéria Renal. Transplante de Rim. Radicais Livres. Ratos.

Introduction

The estimated ratio of patients referred for renal transplantation due to end-stage renal disease in Brazil is 383/1 million, and the numbers are increasing by 9.9% each year. Renal ischemia in donors during the replacement operation is a major cause of acute renal failure[1].

Renal ischemia–reperfusion (I–R) also occurs during several other procedures, particularly renal artery revascularization, suprarenal aortic aneurysm correction and partial nephrectomy, and also during laparoscopic procedures[2,3].

Reperfusion is crucial for the survival of the ischemic tissue. However, it leads to the release and circulation of various species that may cause additional cell damage[4]. I–R of an organ or tissue results in cell damage via a complex cascade of events that affect the structure and function of all cell organelles[4]. Free radicals, prominent mediators of this cell damage, are produced when the oxygen supply is restored through reperfusion[4]. The alterations most commonly seen in these situations are the formation of reactive oxygen species (ROS), depletion of adenosine 5'-triphosphate (ATP), neutrophil infiltration, phospholipase activation and damage to the cell membrane[4].

Ingestion of specific nutrients can modulate the inflammatory response in several organs, thus lowering the damaging force of the physiologic cascade of events[5]. It has been demonstrated that the administration of neutral amino acids, such as glycine and alanine, prevents damage to renal tubule cells from ischemia or hypoxemia[5].

Glutamine (GLN) is the most abundant amino acid found in the bloodstream, and its presence is thought to be essential in the setting of a critical condition[6]. One of its most significant characteristics is to be a precursor of glutathione (GSH), which is considered to be a potent antioxidant agent[7]. It is common knowledge that when the kidneys are submitted to oxidant stress, GLN is the limiting factor for GSH synthesis[7].

Fuller *et al*.[6] reported that GLN administered during the pretreatment of rats undergoing kidney transplantation attenuated tubular cell apoptosis. Mora *et al*.[7] noted partial protection from cisplatin-induced nephrotoxicity in rats treated with GLN. In addition, GLN is known to protect the liver from the effects of post-I–R injury.

In this context, it is most likely that GLN supplementation for 7 days before renal I–R raises the organic antioxidant capacity, with a consequent decrease in renal injury. The scarcity of reports in the literature from studies specifically investigating this has motivated the present research.

The aim of this study was to evaluate the effects of GLN supplementation on the plasma antioxidant system, by assessing: (1) total antioxidant capacity (TAC); (2) glutathione peroxidase; (3) superoxide dismutase (SOD); (4) biochemical markers of blood alterations, such as lactate dehydrogenase (LDH), urea and creatinine; and (5) morphologic repercussions assessed with optic microscopy of renal and lung parenchyma.

Methods

Approved by the Ethics and Animal Research Committee of UNESP (University of Sao Paulo State) under protocol number 463/46/01/10. We used 33 adult male Wistar rats (*Rattus norvegicus albinus*, Rodentia, Mammalia), weighing 280–320 g, from the central laboratory of UFMT (Federal University of the State of Mato Grosso, Brazil). Calculation of sample size, at a minimum of eight animals, took into consideration creatinine level as a marker of renal injury. After a period of environmental adaptation and *ad libitum* administration of standard diet (AIN-1993), all eight animals were kept on a zero solid food diet for 6 hours, after which they were anesthetized with ketamine hydrochloride, 100 mg/kg I.M.

The procedure involved making an incision in the abdominal wall (right flank), opening the abdominal cavity and performing a right-sided nephrectomy. After closure of the abdominal cavity, the animals were returned to the laboratory for postoperative care, remaining on *ad libitum* diet. On the eighth postoperative day, animals were randomized to three groups of 11 animals: group 1, GLN; group 2, albumin (control); and group 3, glucose (sham). Groups received a similar *ad libitum* diet, with particular gavage supplementation in each group, for 7 days. The different diets contained different elements, albeit with similar energy content: in group 1, supplementation was with L-glutamine, 0.5 g/kg orally fed; in group 2 (control), it was with albumin, 0.57 g/kg; and in group 3, it was with 50% concentration glucose, 0.5 g/kg diet. By the end of the 14th day following nephrectomy a new laparotomy was performed on the animals through a broad abdominal incision. The left renal pedicle was carefully dissected in all the three groups; in the GLN and control groups, it was clamped using vascular clamps for 45 minutes (ischemia), after which the renal pedicle of the organ was released for 60 minutes (reperfusion). In the sham group the renal pedicle was repaired for only 45 minutes, without clamping for occlusion, after which the repair was released and the animals kept in the surgical state for another 60 minutes. In all the animals, 5 ml blood samples were collected from the vena cava after 1 hour of reperfusion. The

animals were then killed immediately with halothane inhalation. Kidneys and left lungs were then collected for histological examination, which was performed at the Pathology and Anatomy Service, Julio Muller University Medical School Hospital/UFMT (Figure 1).

Automated biochemical determination of LDH, urea and creatinine levels, TAC, and glutathione peroxidase and SOD levels was performed at Santa Rosa Laboratory (Cuiaba-MT, Brazil), using kits from Randox Laboratories Ltd. All the determinations were made on the same day that the material was collected.

Histological changes were classified using the criteria established by Chander *et al*.[9] for renal injuries, and Greca *et al*.[10] for lung lesions.

Statistical analysis was performed using SPSS for Windows software. The results were analyzed according to their normal distribution using the Ryan–Joiner test and were compared with results using the Shapiro–Wilk test. The non-parametric Kruskall–Wallis test was used to compare the results. Where a statistically significant difference was found, the Mann–Whitney *U* test was used to compare the pairs of results. The statistical significance level was established at 5% ($p<0.05$). Data are presented as means ± standard deviations, or as medians.

Results

All 11 animals in each group (GLN, control and sham) tolerated the experiment well. No animals died during the experiment.

The serum levels of urea were similar in the three groups (GLN group, 70 mg/dl [61–74 mg/dl] mg/dl; sham group 64 mg/dl [53–75 mg/dl]; and control group, 66 mg/dl [56–74 mg/dl]; $p>0.05$). Similarly, serum levels of creatinine did not show any significant differences among the groups (GLN group, 1.05 mg/dl [0.95–1.17 mg/dl]; sham group, 0.96 mg/dl [0.67–1.12 mg/dl]; and control group, 1.03 mg/dl [0.95–1.18 mg/dl]; $p>0.05$).

The LDH level was significantly lower in the sham group (523 U/l [378–736 U/l]) compared with the control (113 U/l [555–1550 U/l]) and GLN (873 U/l [696–1061 U/l]) groups ($p<0.05$). Comparison between the control and GLN groups did not reveal any significant difference ($p=0.23$).

TAC was higher in the GLN (2.22 mmol/ml [2.16–2.36 mmol/ml]) and control (2.22 mmol/ml [2.09–2.33 mmol/ml] groups compared with the sham group (2.10 mmol/ml [2.02–2.21 mmol/ml]), $p<0.05$ (Figure 2).

Glutathione peroxidase levels in the sham (13.365 U/l

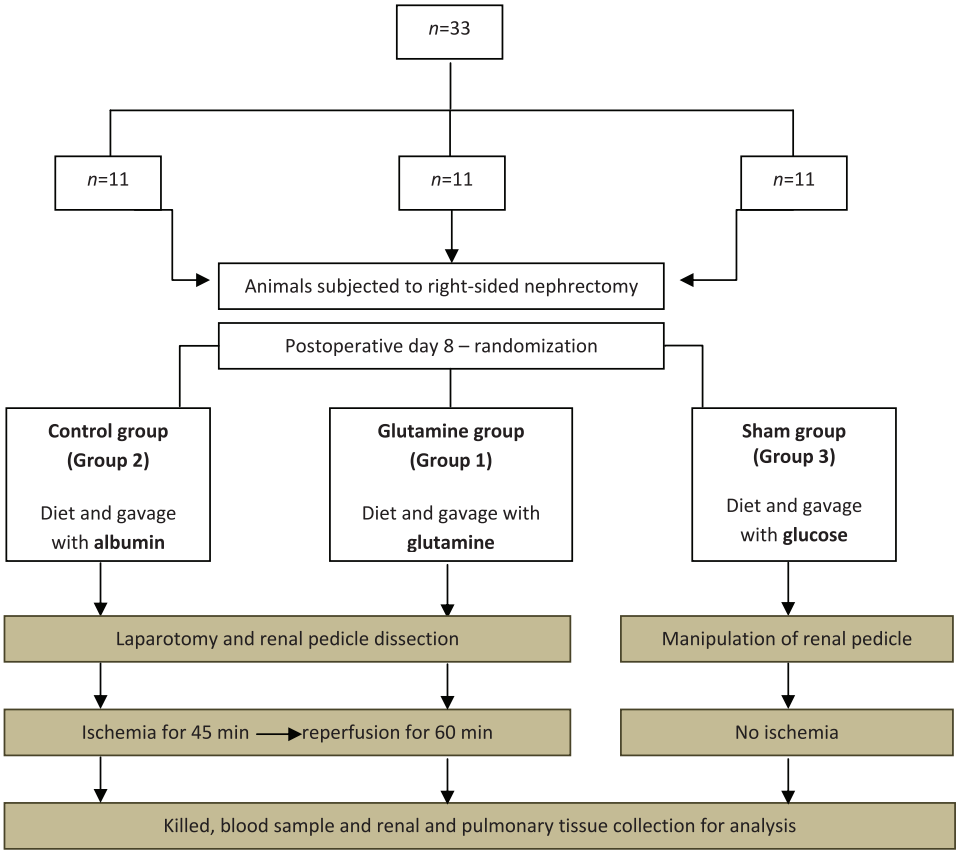

FIGURE 1 - Distribution of experimental animals within the study groups.

[8.440–18.000 U/l]) and GLN (12.439 U/l [9.592–13.154 U/l]) groups were higher when compared with the control group (10.721 U/l [7.975–11.783 U/l]), $p<0.05$. There was no statistically significant difference between the sham and GLN groups (Figure 3).

SOD activity was significantly higher in the sham group (102 U/ml [86–121.2 U/ml]) compared with the GLN (86.4 U/ml [58.8–107.6 U/ml]) and control (86 U/l [54.8–114 U/ml]) groups, $p<0.01$.

The histological study showed fewer morphologic changes in the sham group than in the control and GLN groups. The control and GLN groups, however, had quite similar degrees of histological damage and there was no statistically significant difference between them (Figure 4).

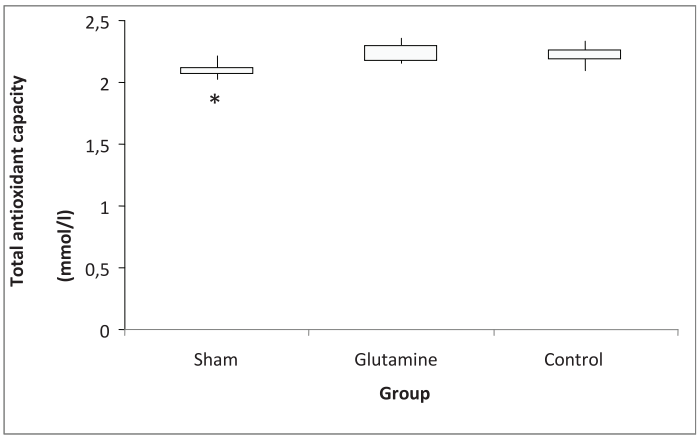

FIGURE 2 - Serum TAC in the three experimental groups. *$p<0.05$ vs GLN and control groups.

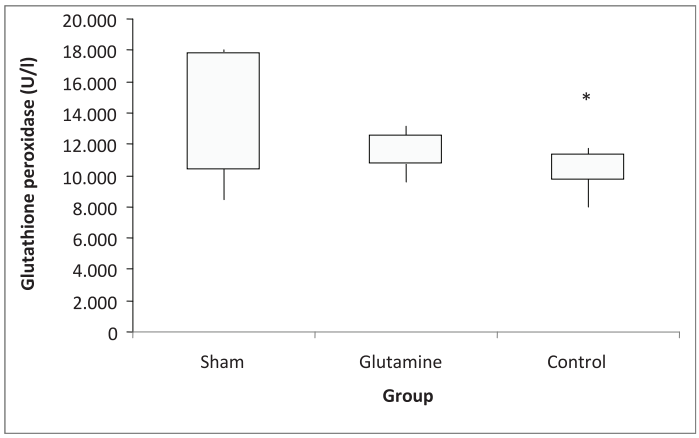

FIGURE 3 - Serum glutathione peroxidase in the three experimental groups. *$p<0.05$ vs GLN and sham groups.

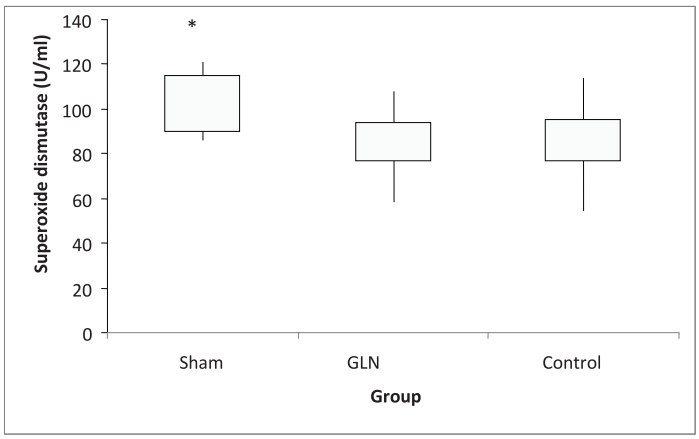

FIGURE 4 - Serum superoxide dismutase in the three groups.*$p<0.05$ vs GLN and control groups.

Discussion

Renal pedicle clamping for vascular control has been used increasingly since it was first described in renal transplantation, partial nephrectomy and aorta aneurysm correction. Additionally, as the complexity of renal surgical procedures has increased – particularly with the advent of videolaparoscopy in urological surgery practice – so too has the use of procedures involving renal ischemia, particularly warm ischemia[2,4,11].

Eschwége *et al*.[21] have demonstrated that a period of renal ischemia longer than 45 minutes causes changes in urea and creatinine levels, with significant elevation after 48 hours of reperfusion and a trend towards normalization after the tenth day. Paller *et al*.[22], in a classic study, observed lower creatinine levels in rats in which SOD had been administered before 1 hour of renal ischemia followed by reperfusion for 15 minutes than in rats that had not received SOD. Such differences were more striking in samples collected after 24 hours of ischemia.

Tucci *et al*.[23] demonstrated protection with chlorpromazine administration when renal ischemia was applied for 1 hour; however, urea and creatinine levels rose after 24 hours of reperfusion.

In the present study no difference in urea and creatinine results was observed among the groups. This finding may be related to the reperfusion period, which was only 60 minutes. Additionally, compensatory renal hypertrophy, a phenomenon already well established in the literature may have occurred[24].

In our study, renal ischemia was related to a significant increase in LDH in the GLN and control groups when compared with the sham group. Although GLN had a protective effect, this did not reach statistical significance. This has also been observed in other studies on oxidative stress, in which the benefit from using

GLN could not be proven[15].

Acidosis (one of the main components of I–R syndrome) was indirectly demonstrated by the greater rise in LDH values in the control group. In the literature we found an association of acidosis with hyperkalemia, myoglobinemia, myoglobinuria, acute renal failure, non-cardiogenic pulmonary edema and alterations of the pressor response, all of which resulted in multiple organ dysfunction and death[25]. In this study these other systemic parameters of I–R syndrome were not assessed; therefore, there is only a suggestion that other organs may have been involved in the resulting damage. Similarly, there is a suggestion that GLN, acting at the cellular level, might have acted as a protective factor.

In our study, we found similar glutathione peroxidase values in the sham and GLN groups; the control group had significantly lower levels. These results indicate that animals that received GLN supplementation had higher GSH reserves, as levels were maintained despite the predicted expenditure in terms of tamponade oxidative radicals generated by renal I–R. This finding seems to corroborate the beneficial effect of GLN as a cell-protective agent in I–R.

This effect was also observed by Ezhilan *et al*.[12] in myocardial cells subjected to oxidative injury. Their conclusion was that elevation of total GSH and glutathione peroxidase levels was due to previous treatment with GLN[12]. Abilés *et al*.[13] demonstrated an increase in glutathione peroxidase when supplementing patients with severe disease with GSH, and this increase was proportional to the elevation of GSH; they also found an inverse relationship between levels of this enzyme and lipid peroxidation in these patients. A similar result was reported by Kul *et al*.[14], who used enteral GLN in animal models, with elevation in glutathione peroxidase levels and a decrease in lipid peroxidation in the bowel.

In the model used in this study, GLN was shown to preserve total plasma antioxidant capacity. Such an effect has already been demonstrated by other authors such as Bicudo *et al*.[15] and Szijártó *et al*.[16] in animal models of hepatic I–R. This may be related either to a rise in plasma antioxidant components, with – once again – GSH prominent, or to an inhibitory action on ROS production, consequently decreasing the organic demand for antioxidants. An interesting observation was that TAC values were similar in the GLN and control groups. This might be explained by albumin also having an antioxidant capacity, as reported by other investigators[17,18].

SOD activity was reduced in the GLN and control groups in comparison with the sham group. This behavior is compatible with results from Ezhilan *et al*.[12] Additionally, Jia *et al*.[19] did not observe elevated SOD levels after hepatic I–R in rats that had received GLN prior to an I–R event.

Ahmet, *apud* Kahraman[20] suggests that SOD is inactivated by high levels of oxygen radicals, their amounts decreasing after I–R. Alternatively, there may have been high expenditure of this enzyme during the reperfusion phase. This is a possibility, as SOD is a key antioxidant enzyme that neutralizes the superoxide radical, a molecular precursor of all other ROS and their derivatives.

Kramer *et al*.[26] highlighted the increase in pulmonary vascular permeability after renal I–R. This occurred after only 1 hour of renal reperfusion, with a peak occurring at 48 hours of reperfusion, and the effect disappearing after 96 hours.

Deng *et al*.[27] detected evidence of alterations in pulmonary histology after 4 hours of renal reperfusion. These comprised edema in the interstitial spaces, increases in interstitial cellularity and inflammation of the alveolar space.

In our study the pulmonary and renal histological changes were of discrete intensity, both in the GLN and control groups, and were substantially less intense in the sham group. This justifies with the fact that reperfusion was not extended for a longer period of time. Eventually, in the histological study a longer reperfusion could determine the same benefit seen in the systemic antioxidant activity with ingestion of this amino acid.

In conclusion, this study using GLN proposes a novel approach that may ultimately benefit patients undergoing surgical procedures involving renal I–R, as well as those undergoing renal transplantation. We must pay attention to the fact that recent studies propose ischemic preconditioning in organs to be subjected to I–R stress. Such preconditioning requires induction with a short period of ischemia followed by a short reperfusion period, before the longer ischemia occurs. The role of ischemic preconditioning in an enhanced tolerance to ischemia has been reported for several organs, such as the heart, brain, spinal cord, skeletal muscle, retina, kidneys, bowel and the liver. However, despite the relative abundance of studies on the protective effects of ischemic preconditioning, the physiological foundations of this approach have not yet been clearly established[28].

Conclusion

The administration of glutamine prior to a renal ischemia–reperfusion improved the antioxidant system, as it was associated with preservation of total antioxidant capacity and elevation of glutathione peroxidase, even in the absence of improvement in other study variables, including kidney and pulmonary histology.

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