Renal ischemia and reperfusion injury: influence of chorpromazine on renal function and lipid peroxidation[1]

Lesão de isquemia e reperfusão renal: influência da clorpromazina na função renal e na peroxidação lipídica

Silvio Tucci Junior[I], Roberto Marins de Carvalho[II], Fábia Martins Celini[III], Adauto José Cologna[IV], Haylton Jorge Suaid[V], Luis Fernando Tirapelli[VI], Antonio Carlos Pereira Martins[VII]

I MD, PhD, Associated Professor, Division of Urology, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
II MD, Fellow PhD degree, Division of Urology, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
III Fellow Master degree, Division of Urology, Department of Pediatric, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
IV MD, PhD, Division of Urology, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
V MD, PhD, Associated Professor, Division of Urology, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
VI MD, PhD, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.
VII Full Professor and Head, Division of Urology, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.

ABSTRACT

Purpose: To evaluate the influence of chlorpromazine (CPZ) on renal function and lipid peroxidation in a rat model of kidney ischemia/reperfusion injury. **Methods:** Forty eight Wistar rats underwent a laparotomy for hilar clamping of left kidney with a bulldog clamp for 60 minutes followed by organ reperfusion and contralateral nephrectomy. Of these, 26 received 3mg/kg of CPZ intravenously 15 minutes before renal ischemia (G-E) while the remaining 22 were used as ischemic control group (G-C). Eleven rats of G-E and 8 of G-C were followed for blood urea nitrogen and creatinine determinations before renal ischemia and at 1st, 4th and 7th postoperative days. Samplings of left renal tissue were obtained at 5 minutes (5 rats from each group) and 24 hours (9 G-C and 10 of G-E) of reperfusion for malondialdehy (MDA) content determination. Controls of renal MDA content were determined in kidneys harvested from 6 additional normal rats. **Results**: Acute renal failure occurred in all animals but levels of BUN and creatinine were significantly lower in G-E ($p<0.001$). MDA content rose strikingly at 5 minutes of reperfusion in both groups ($p>0.05$) and returned near to normal levels 24 hours later. **Conclusion**: CPZ conferred partial protection of renal function to kidneys submitted to ischemia/reperfusion injury that seems to be not dependent on inhibition of lipid peroxidation.

Key words: Renal Ischemia/Reperfusion. Lipid Peroxidation. Malondialdehyde. Chlorpromazine. Free Radicals.

RESUMO

Objetivo: avaliar a influência da clorpromazina (CPZ) na função renal e na peroxidação lipídica num modelo de lesão de isquemia/reperfusão renal em ratos. **Métodos:** 48 ratos Wistar foram submetidos à laparotomia para clampamento da artéria renal esquerda durante 60 minutos, seguido da reperfusão e nefrectomia contralateral. Destes animais, 26 receberam 3 mg/kg de CPZ intravenosa 15 minutos antes da isquemia renal (G-E), sendo os 22 animais restantes utilizados como grupo controle isquêmico (G-C). Em 11 ratos do G-E e 8 do G-C foi feita a dosagem de uréia e creatinina sérica antes da isquemia renal e no 1º, 4º e 7º dia pós-operatório. Amostras de tecido do rim esquerdo foram obtidas aos 5 minutos (5 ratos de cada grupo) e 24 horas após reperfusão (9 G-C e 10 G-E) para dosagem de malondialdeído (MDA). Valores controle para níveis de MDA foram obtidos em rins retirados de 6 ratos normais. **Resultados:** insuficiência renal aguda ocorreu em todos os animais mas os níveis séricos de uréia e creatinina foram significativamente menores no G-E ($p<0,001$). Os níveis de MDA apresentaram elevação acentuada na avaliação aos 5 minutos de reperfusão em ambos os grupos ($p<0,05$), retornando a valores próximos aos normais na avaliação com 24 horas. **Conclusão:** a CPZ conferiu proteção parcial da função renal aos rins submetidos à lesão de isquemia e reperfusão, aparentemente independente da inibição da peroxidação lipídica.

Descritores: Isquemia/Reperfusão Renal. Peroxidação Lipídica. Clorpromazina.

1. Research performed at Department of Surgery and Anatomy, Division of Urology, Ribeirão Preto Faculty of Medicine, University of São Paulo (USP), Brazil.

Introduction

Ischemia/reperfusion (I/R) injury is a major cause of acute renal failure occurring after hemorrhagic shock or major cardiovascular surgery, and the most common cause of delayed graft function or organ failure.[1,2] Ischemia-reperfusion injury is a complex phenomenon involving not only intracellular processes but also an injurious inflammatory response that are interconnected.[3] While the pathogenesis of ischemia-reperfusion is not completely understood, there is considerable evidence implicating the decreased mitochondrial ATP generation, loss of selective permeability of cell membranes, impairment of cellular ion homeostasis (rise of intracellular sodium and calcium) with activation of hydrolases and accumulation of reactive oxygen species (ROS) leading to cell injury or death.[3-5] ROS formed during oxidative stress can initiate lipid peroxidation, oxidize proteins to inactive states and cause DNA strand breaks, all potentially damaging to normal cellular function.[3,4] Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes, and their destruction can lead to the production of toxic reactive aldehydic metabolites and cell death.[5-8] A range of drugs and methods have been tested with the intention to block the pathways of cell injury at different levels and to thereby enhance viability of the kidney challenged by ischemia/reperfusion stress with variable results, such as: heat shock (interferes with the L-arginine-nitric oxide pathway),[9] antioxidants,[10,11] dipyridamole (increases endogenous adenosine),[12,13] nicotine (inhibit cholinergic anti-inflammatory pathway),[14] calcium channel blockers or antagonists,[15-18] chlorpromazine,[19,20] etc. Chorpromazine (CPZ) is a membrane stabilizer and seems to be able to preserve mitochondria integrity and to reduce phospholipids degradation of microsomal membranes of liver cell provoked by ischemia/reperfusion.[20,21] The aim of our study was to explore the effect of chlorpromazine on renal function and membrane lipid peroxidation in a rat model of renal ischemia/reperfusion.

Methods

Forty eight Wistar *Rattus novergicus*, weighting 200-250g, were anesthetized with an intraperitoneal injection of thiopental (20mg/kg) and underwent a laparotomy followed by dissection of the left kidney and hilar clamping with a bulldog clamp for 60 minutes followed by organ reperfusion and contralateral nephrectomy. Of these, 26 received 3mg/kg of chlorpromazine intravenously 15 minutes before renal ischemia (Group E) while the remaining 22 were used as ischemic control group (Group C). Eleven rats of Group E and 8 of Group C were followed for blood urea nitrogen (Urease-Labtest Cat 27®) and creatinine (Creatinine-Labtest Cat 35®) determination before renal ischemia and in the 1st, 4th and 7th postoperative days. Samplings of left renal tissue were obtained at 5 minutes (5 rats from each group) and 24 hours (9 of Group C and 10 of Group E) of reperfusion for malondialdehy (MDA) content determination. Normal controls of renal MDA content were determined in left kidneys harvested from 6 additional normal rats. For tissue sampling in the described moments and at the end of experiment the animals were sacrificed by decapitation.

For the determination of MDA content kidney samples were homogenized in 9 volumes of Tris-HCl 10mM, pH 7.4, and centrifuged at 3,000 G for 10 minutes at 4ºC. The supernatant was aspirated and used to quantify MDA (expressed in μM) using a lipid peroxidation assay kit (Calbiochem, cat # 437634, Novobiochem Co, USA) and a spectrophotometer at absorbance of 586nm.

Statistical analyses were performed with the software program GraphPad Prism 4 (GraphPad Software Inc, San Diego, CA). Continuous variables among groups were compared by Kruskal-Wallis method and Dunn´s multiple comparisons test as long as their distribution did not pass the normality test or the differences among the SDs were very significant. A level of $P<0.05$ was set as statistical significant.

Results

The results of blood urea nitrogen (BUN) and creatinine in groups C and E at different moments of the experiment are displayed in Tables 1 and 2. In the group C renal dysfunction lasted till the 7th postoperative day and in all moments was worse than in group E ($p<0.001$). In the group E, creatinine and BUN returned to normal levels on the 7th postoperative day.

TABLE 1 – Blood urea nitrogen (mean±SD; mg/dl) at different moments from ischemia in control (without chlorpromazine) and experimental (with chlorpromazine) groups.

Group	Before ischemia	1 day*	4 days*	7 days*	P value♣
Control	61.3±4.7	240.0±28.6	239.4±80.6	135.5±64.9	<0.001
Experimental	60.4±14.8	107.8±29.7	76.8±27.9	59.4±9.1	<0.005
P value♣♣	>0.05	<0.001	<0.001	<0.001	

* Time after ischemia; ♣ Kruskal-Wallis test; ♣♣ Dunn's multiple comparisons test

TABLE 2 – Serum creatinine (mean±SD; mg/dl) at different moments from ischemia in control (without chlorpromazine) and experimental (with chlorpromazine) groups.

Group	Before ischemia	1 day*	4 days*	7 days*	P value♣
Control	0.6±0.1	3.4±0.7	2.7±1.4	1.4±0.6	<0.001
Experimental	0.6±0.04	1.2±0.3	1.0±0.3	0.6±0.1	<0.001
P value♣♣	>0.05	<0.001	<0.001	<0.001	

* Time after ischemia; ♣ Kruskal-Wallis test; ♣♣ Dunn's multiple comparisons test

The mitochondrial MDA content found in normal kidneys and in kidneys challenged with 60 minutes of ischemia followed by 5 minutes or 24 hours of reperfusion is expressed in Table 3. The MDA rose significantly at 5 minutes of reperfusion in both groups of rats, treated or not with CPZ (p>0.05). Twenty four hours after the ischemic challenge MDA content tended to return to pre-ischemic values.

TABLE 3 – Effect of pretreatment with chlorpromazine (CPZ) on malondialdehyde content (μM) of mitochondria supernatants derived from normal and reperfused kidneys after 60 minutes of warm ischemia.

Renal condition	N	Malondialdehyde** (Mean±SD)
No ischemia – control (a)	6	4.0±0.3
5 minutes of reperfusion		
- without CPZ (b)	5	7.4±2.1
- with CPZ (c)	6	9.4±1.9
24 hours of reperfusion		
- without CPZ (d)	9	4.8±0.7
- with CPZ (e)	10	5.8±0.8
P value (all groups)*		<0.0001

*Kruskal-Wallis test; **Significant p values calculated by Dunn's test: a x b=<0.01; a x c=<0.001; a x e=<0.05; c x d=<0.01

Discussion

Kidneys submitted to warm ischemia during 60 minutes exhibit functional deficit resulting in uremia as published previously by others.[22,23] In the control group the BUN and creatinine levels remained high till the 7th postoperative day even though exhibiting a tendency to return to normal values. In the group treated with CPZ the levels of BUN and creatinine were lower than in the control group in the period 1st- 4th days, and at the 7th day their values return to normal. Thus, CPZ in the dose of 3mg/kg conferred a partial protection of renal function to kidneys challenged with warm ischemia for 60 minutes. CPZ ability to protect tissues against ischemia/reperfusion was reported by others working with several organs such as: kidney,[22,24] liver,[19,25] nervous system,[26] heart.[27] Upon ischemia/reperfusion challenge, organ depletion of ATP causes mitochondrial dysfunction, and accumulation of intracellular sodium, calcium and ROS.[4] An associated mechanism of cell injury is the accelerated rate of phospholipid degradation in ischemic rat liver tissue, producing a loss of almost one half the total cellular phospholipid with 3 hours of ischemia, and pretreatment of the rats with CPZ (20mg/kg) completely prevented the disturbed phospholipid metabolism at the same time that it prevented the cell death associated with as much as 3 hours of ischemia.[19,28] Several studies have also demonstrated that warm ischemia in kidney is associated with lipid peroxidation that leads to oxidative destruction of cellular membranes, and their destruction can lead to the production of toxic, reactive aldehydic metabolites and cell death.[1,7,8] Among the aldehydic metabolites, MDA and 4-hydroxynonenal are the most important.[29,30] Superficial and deep cortex immunostaining with antibody against MDA was observed after 30 minutes of warm renal ischemia and such immunostaining was observed in the absence of any histological lesions, as assessed by routine staining; after 45 and 60 minutes of warm ischemia, lipid peroxidation byproducts were detected both in the cortex and in the medulla, which is associated with 33% and 66% of rat deaths respectively.[31] Thus the lipid peroxidation process is involved in kidney damage during anoxia before reperfusion, and the presence of its byproducts in renal medulla was associated with the animals' death.[31] Our study confirms that lipid peroxidation occurs during anoxia since MDA content rose strikingly at 5 minutes of kidney reperfusion and tended to return to normal values 24 hours later. However, pretreatment of animals with CPZ, dose of 3mg/kg, was not able to inhibit lipid peroxidation in our model of renal ischemia which is in disagreement with the findings reported for ischemic rat liver.[19,28] A possible explanation for such controversy may be based on the difference of CPZ dose employed that was of 20mg/kg in the model of liver ischemia. The protection of renal function against ischemia/reperfusion injury promoted by CPZ at a dosage of 3mg/kg seems to be not related to inhibition of lipid peroxidation.

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Correspondence:
Silvio Tucci Junior
Department of Surgery and Anatomy
Ribeirão Preto Faculty of Medicine, University of São Paulo
14.048-900 Ribeirão Preto, São Paulo, Brazil.
Phone/Fax: (+55) 16 3602-2593
stucci@convex.com.br

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Comments:
In recent years, as pointed by Tucci Jr and colleagues, numerous studies have been conducted on the deleterious effects of reperfusion following ischemia in different organs including the kidney. Among these studies there are strong evidences that formation of oxygen-derived free radicals and subsequent lipid peroxidation are implicated as the causative factors of these injuries. There are many reports about the usefulness of antioxidant drugs as well as the application of methods such as ischemic preconditioning in the prevention of oxidative stress induced-injury. Also, has been proved that nitric oxide is involved on the pathophysiology of renal ischemia reperfusion injury, justifying the use of nitric oxide donors as renal failure complimentary treatment. Chlorpromazine is an alpha-blocker vasodilator widely used in the seventies, to treat renal failure, but there are few studies taking in account its mechanisms of action concerning lipid peroxidation. One good study[1] refers that chlorpromazine has antioxidant effects, is a putative cellular phospholipase inhibitor and calmodulin antagonist, blocking the increase in MDA formation. The injury was caused by renal proximal tubule cell death induced by haloalkene cysteine conjugates. In the present study Tucci Jr and colleagues, using a rat model of renal ischemia reperfusion injury, chlorpromazine had a protection effect apparently independent of lipid peroxidation, since MDA values are high in both groups. The study has a good design and, in my opinion, it is possible to speculate if the lipid peroxidation has different mechanisms according renal injury cause (chlorpromazine blocks the oxidative stress in the haloalkene cysteine conjugates model and does not block it in case of ischemia reperfusion injury).

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Paulo Roberto B. Evora
Full Professor and Head, Division Thoracic and Cardiovascular Surgery, Department of Surgery and Anatomy, Ribeirão Preto Faculty of Medicine, University of São Paulo, SP, Brazil.

Comments:
Studies on the several events occurring during ischemia/reperfusion are certainly important, especially when correlated to physiopathologic alterations in organ transplants. In addition, drugs that may "protect" the renal tissue from severe lesions, which extend kidney regeneration post-transplant, are helpful in clinical applications. In this experimental study, 48 Wistar rats were submitted to renal artery occlusion of one kidney for 60 minutes, followed by contra lateral nephrectomy. Of these animals, 26 received an endovenous injection of chlorpromazine 15 minutes before renal ischemia by artery occlusion and the remaining ones were kept as controls. Samples of renal tissue, removed 5 minutes and 24 hours after perfusion were analyzed for determination of malondialdehyde (to measure lipid peroxidation in the model utilized) Acute renal failure (ARF) was seen in all animals, but plasma levels of urea and creatinine were lower in the ones chlorpromazine treated. Alterations of malondialdehyde tissue levels were not different in both groups in the study. It seems that the partial chlorpromazine protection of renal function in kidneys submitted to the ischemic lesion, is independent of lipid peroxidation. Ischemia/reperfusion physiopathology is not completely established, but experimental models like the one utilized may answer some questions and eventually supply information on the clinical use of drugs as chlorpromazine. The authors suggest that higher drug dosages may produce different results in the same model.

Miguel Moyses Neto
MD, PhD, Division of Nephrology, Department of Clinical Medicine, Ribeirão Preto Faculty of Medicine, University of São Paulo, Brazil.