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Bupropion Cannot Reduce Acute Kidney Injury Due to Ischemia-Reperfusion

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Abstract

Bupropion can limit the increase of TNF-alpha, reducing the inflammatory response and injury due to Ischemia-Reperfusion (I/R) on tissues such as the bowel. Our objective was to evaluate bupropion as a preconditioning agent for ischemic acute kidney injury.

Experiments were performed on female Wistar rats. Bupropion groups received 25 mg/kg 60 min before the maneuver. As a first step, 30 rats with a right nephrectomy were divided into 6 groups (n=5): sham 24, sham 48, bupropion 24, bupropion 48, ischemia-reperfusion 24 and ischemia-reperfusion 48. After 60 min of ischemia (except sham groups) 24 or 48 h of reperfusion was allowed. During a second step, 30 rats, with both kidneys conserved, were divided into 6 groups (n=5): sham 3, sham 72, bupropion 3, bupropion 72, ischemia-reperfusion 3, and ischemia-reperfusion 72 (IR72). Ischemia in these groups (except sham groups) was performed for 60 min only on the left kidney, and 3 or 72 h of reperfusion were allowed. Serum and histologic evaluations were done.

After 48 h of reperfusion, all mono-renal rats that received bupropion died. Bupropion cannot avoid histological damage nor does it impact creatinine clearance, BUN, TNF-α or KIM-1 serum levels secondary to I/R. In conclusion: The pharmacologic preconditioning with Bupropion cannot improve the AKI evolution in rats with renal ischemia-reperfusion injury.

Keywords: Acute kidney injury; Preconditioning kidney; Bupropion, Ischemia reperfusion injury

Introduction

Acute Kidney Injury (AKI) can complicate the evolution of kidney transplant recipients as well as diseases frequently related to the reduction or interruption of renal blood flow, such as renal trauma or hemorrhagic shock [1,2]. AKI can cause permanent loss of kidney function [3], has a poor prognosis, and a high mortality rate (20% to 35%). When it is associated with hemodynamic alterations, mortality can increase up to 60% [4,5].

During the early phases of AKI an acute inflammatory process develops, and affects epithelial and endothelial cells [6]. This process is organized by tumor necrosis factor-alpha (TNF-α); one of the main mediators of renal ischemia/reperfusion (I/R) injury and graft rejection [7]. TNF-α promotes leukocyte infiltration, induces cell death and endothelial dysfunction, mediates remote organ injury, and activates an inflammatory cascade that results in further production of cytokines and chemokines. The pharmacologic modulation of TNF-α production has been a strategy in the attempt to prevent I/R injury [4].

Bupropion is an antidepressant whose main mechanism of action is the inhibition of dopamine and noradrenalin reuptake. The increased signalling at beta-adrenergic and D1 receptors result in an increase of cAMP that inhibits TNF-α synthesis. It also decreases leukocytes,

serum levels of pro inflammatory cytokines such as interferon-γ and Interleukin-1, and elevates the anti-inflammatory interleukin-10 [8].

Previous studies have shown that bupropion can reduce the inflammatory response [9], and animal intestinal I/R models have demonstrated a decrease in TNF-α [10]. To our knowledge there are no studies that evaluate the effect of bupropion as a pharmacologic preconditioner in renal I/R. Our objective was to investigate if bupropion could reduce renal I/R injury during AKI.

Materials and Methods

Experiments were performed on 60 female Wistar rats weighing between 200-250 g. Animals were maintained under standard conditions with access to commercial rat pellets and water ad libitum. All animal procedures were performed in accordance with the proper use and care of laboratory animals and approved by our ethics committee (IRB approval number R-2013-785-059 and FI13-003).

The experiment was carried out in two stages. The first stage included thirty unilateral nephrectomised rats that were randomly divided into 6 groups (n=5): sham 24 (SH24), sham 48 (SH48), bupropion 24 (BUP24), bupropion 48 (BUP48), ischemia-reperfusion 24 (IR24) and ischemia-reperfusion 48 (IR48). A second stage included thirty rats with kidneys conserved and I/R produced in the left kidney. These were randomly divided into 6 groups (n=5): sham 3

(SH3), sham 72 (SH72), bupropion 3 (BUP3), bupropion 72 (BUP72), ischemia-reperfusion 3 (IR3) and ischemia-reperfusion 72 (IR72).

The rats were anesthetized with ketamine/xylazine (Anesket, Pfizer Inc., Mexico) at a intraperitoneal dose of 50/10 mg/kg, before performing laparotomy. 60 min before the surgery the BUP groups received a dose of bupropion at 25 mg/kg (Wellbutrin, GlaxoSmithKline) P.O. via oral gavage as described by Brustolim et al. [8], while the SH and IR groups received placebo.

In the BUP and IR groups ischemia was induced using a non-traumatic microvascular clamp that occluded the left renal pedicle for 60 min. This procedure obstructed both renal vein and artery, confirming ischemia by changes in renal coloration and loss of arterial pulse. In the SH groups, the left kidney was only manipulated without clamping the renal pedicle.

After the clamps were removed, reperfusion was allowed for 24 h (SH24, BUP24 and IR24), 48 h (SH48, BUP48 and IR48), 3 h (SH3, BUP3 and IR3) or 72 h (SH72, BUP72 and IR72). In the 24 or 48 h' groups, the right kidney were removed before the abdominal wall and skin were sutured.

After the reperfusion period, rats were euthanized by exsanguination from the aorta under anaesthesia. The blood samples were then centrifuged at 3,500 rpm for 15 min and serum was collected and stored at −70°C for further analysis.

Serum analysis

Serum samples were used to determine serum levels of creatinine, Blood Urea Nitrogen (BUN), Alanine Transaminase (ALT), and Aspartate Aminotransferase (AST) using commercially available standard automated biochemical methods on a Vitros DT60 II Analyser (Johnson and Johnson, New Brunswick, NJ). Serum concentrations were measured for TNF-α using a rat TNF-α ELISA kit (Peprotech Mexico, SA de CV., Mexico City, Mexico), Kidney Injury Molecule-1 (KIM-1) using a Rat KIM-1 ELISA Kit (Abcam plc., Cambridge, UK), and Neutrophil Gelatinase-Associated Lipocalin (NGAL) using a rat Lipocalin-2 (NGAL) ELISA Kit (Abcam).

Tissue examination

The rats were perfused after exsanguination with a buffer solution for 10 min, followed by 10% neutral buffered formalin for another 10 min. Left kidneys were collected, divided by sagittal diameter, fixed in 10% formalin overnight and embedded in paraffin to obtain 4-mm-thick sections that were stained with haematoxylin and eosin for light microscopy. These were examined by a blinded pathologist using the Jablonoski score [11] for kidney injury to evaluate the degree of histologic damage. This score consists of a five-point scale: 0-normal, 1-necrosis of individual and mitotic cells in renal tubular necrosis, 2-necrosis of a group of proximal convoluted tubules in renal tubular necrosis, 3-necrosis of distal third of the proximal convoluted tubules in renal tubular necrosis, 4-necrosis of all parts of the proximal convoluted tubule in renal tubular necrosis.

Statistical analysis

Normal distribution of the study variables were examined with the Shapiro-Wilk test. The variables were analysed using a one-way analysis of variance and the Tukey or T3-Dunnet post hoc test or the Kruskal-Wallis and Mann-Whitney U test. All results are expressed as mean values ± standard deviations with a $P < 0.05$ considered statistically significant.

Results

Monorrenal rats

Mortality and histological damage: Unilateral nephrectomy did not affect the survival of animals, except in the group that received Bupropion before the event of I/R (BUP48), which died within 48 h. Tubular epithelium injury secondary to I/R graded by Jablonosky score, increased significantly 24 and 48 h after ischemia (0.2 ± 0.4 vs. 3.0 ± 0.0 and 0 ± 0 vs. 2.40 ± 0.5; respectively, p<0.05). The BUP24 group did not differ with the positive control group (IR24) (Table 1).

	SH24	IR24	BUP24	SH48	IR48	BUP48
Injury score	0.2 ± 0.4	3.0 ± 0.0[*], [**], [***]	2.4 ± 0.8[*], [***]	0 ± 0	2.40 ± 0.5[*], [***]	
BUN (mg/dL)	22.2 ± 2.8	89.2 ± 58.8	66.2 ± 65.5	21.8 ± 3.9	142.8 ± 90.1[*]	–
Creatinine (mg/dL)	0.78 ± 0.5[*]	1.80 ± 1.3[*], [**]	1.34 ± 1.2[*], [**]	0.48 ± 0.04	4.0 ± 2.0[*]	
Creatinine clearance (mL/min)	1.3 ± 1.9	0.2 ± 0.2[*]	0.4 ± 0.09[*]	2.1 ± 1.3	0.29 ± 0.3[*]	–
AST (U/L)	271.8 ± 151.8	226.2 ± 58.0	176.8 ± 37.9	130.0 ± 6.1	292.6 ± 188.7	
ALT (U/L)	49.2 ± 5.8	33.4 ± 5.7	49.0 ± 10.6	39.8 ± 10.9	53.8 ± 41.6	
TNF-α (ng/mL)	0.61 ± 0.3[*]	0.5 ± 0.6[*], [**]	0.9 ± 0.5	1.3 ± 0.4	3.7 ± 4.3	

Table 1: Kidney injury score, functional, unspecific cellular injury and inflammation biomarkers related with ischemia, after 24 or 48 h of reperfusion on monorrenal rats. BUN: Blood Urea Nitrogen; AST: Aspartate Aminotransferase; ALT: Alanine Transaminase ; TNF-α: Tumour Necrosis Factor-alpha,[*] p<0.05 vs. SH48,[**]p< 0.05 vs. IR48, [***] p< 0.05 vs. SH24.

Functional test: In monorrenal rats, serum levels of BUN, creatinine, and creatinine depuration were used to determine renal function. BUN level increased with the time of reperfusion, differing to 48 h of the SH24 (22.2 ± 2.8 vs. 142.8 ± 90.1 mg/dL; p<0.05). The I / R, also increased creatinine levels significantly within 48 h (1.80 ± 1.3 vs. 4.0 ± 2.0; IR24 vs. IR48, p<0.05), while creatinine clearance decreased after 48 h from I/R injury 2.1 ± 1.3 to 0.29 ± 0.3 (p<0.05). Since none of the BUP48 survived, only the BUP24 group was analysed; There were not differences between BUP24 and IR24 (Table 1).

Unspecific cell injury and Inflammation: ALT and AST were used to monitor nonspecific cell damage. No significant differences were found on nephrectomised rats at 24 or 48 h (SH24, SH48, IR24, IR48, and BUP24). The inflammatory process was monitored using TNF-α. Serum levels were increased significantly 48 h after surgery on monorrenal rats including the Sham group (Table 1).

Non-nephrectomized rats, unilateral ischemia

Mortality and histological damage: No deaths occurred in the non-nephrectomized rat groups. Sham groups were similar between themselves. In the BUP groups, I/R significantly increased histological injury compared to Sham: 1.2 ± 0.45 vs. 2.0 ± 0 and 0.4 ± 0.55 vs. 3.0 ± 0 ($p>0.05$); with three and 72 h of reperfusion time. However, BUP groups had no significant difference with the positive control group (I/R) after 3 or 72 h (Table 2 and Figure 1).
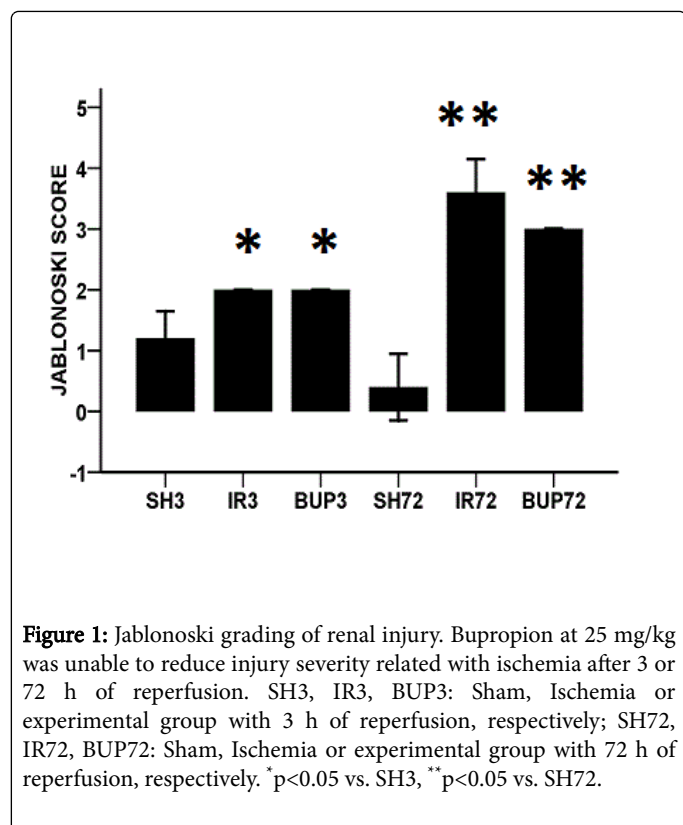

Figure 1: Jablonoski grading of renal injury. Bupropion at 25 mg/kg was unable to reduce injury severity related with ischemia after 3 or 72 h of reperfusion. SH3, IR3, BUP3: Sham, Ischemia or experimental group with 3 h of reperfusion, respectively; SH72, IR72, BUP72: Sham, Ischemia or experimental group with 72 h of reperfusion, respectively. *$p<0.05$ vs. SH3, **$p<0.05$ vs. SH72.

	SH3	IR3	BUP3	SH72	IR72	BUP72
Injury Score	1.2 ± 0.45	2.0 ± 0*	2.0 ± 0*	0.4 ± 0.55	3.6 ± 0.55**	3.0 ± 0**
TNF-α (ng/mL)	2.52 ± 0.8	2.18 ± 1.58	1.94 ± 1.12	0.17 ± 0.27*	0.49 ± 0.71*	0*
KIM (ng/mL)	186.5 ± 68.6	176.6 ± 19.0	257.9 ± 41.3**	82.9 ± 55.7	555.9 ± 223.7***	236.2 ± 60.1***
NGAL (ng/mL)	577.9 ± 132.1	728.8 ± 102.6	741.6 ± 96.4	—	—	—

Table 2: Kidney injury score, inflammation and renal injury biomarkers related with unilateral ischemia, after 3 or 72 h of reperfusion. TNF-α: Tumor Necrosis Factor-alpha; KIM-1: Kidney Injury Molecule-1; NGAL: Neutrophil Gelatinase-Associated Lipocallin, *$p<0.05$ vs. SH3, **$p<0.05$ vs. SH72, ***$p=0.06$ vs. SH72.

Inflammation and specific cell injury: While in rats with right nephrectomy and I/R, a significantly increase in TNF-α to 48 h was

identified (0.5 ± 0.6 vs. 3.7 ± 4.3; $p<0.05$), in animals which retained both kidneys it was not possible to identify differences between the first 3-72 h, sera levels of TNF-α in all groups (SH, I/R, and BUP) were similar and the levels were significantly lower at 72 h in all the groups. KIM-1 was used as a serum marker for specific tubular injury. It increased at 72 h of reperfusion on the animals subjected to ischemia 555.9 ± 223.7 and 236.2 ± 60.1 ng/mL, IR72, BUP72, respectively; however, it was not statistically significant ($p=0.06$ vs. SH72) and despite the fact that KIM-1 is less in the BUP72 group, it was not different from the I/R72, with lower levels. Serum NGAL levels were not different between the groups at 3 h or reperfusion (Table 2 and Figure 2).
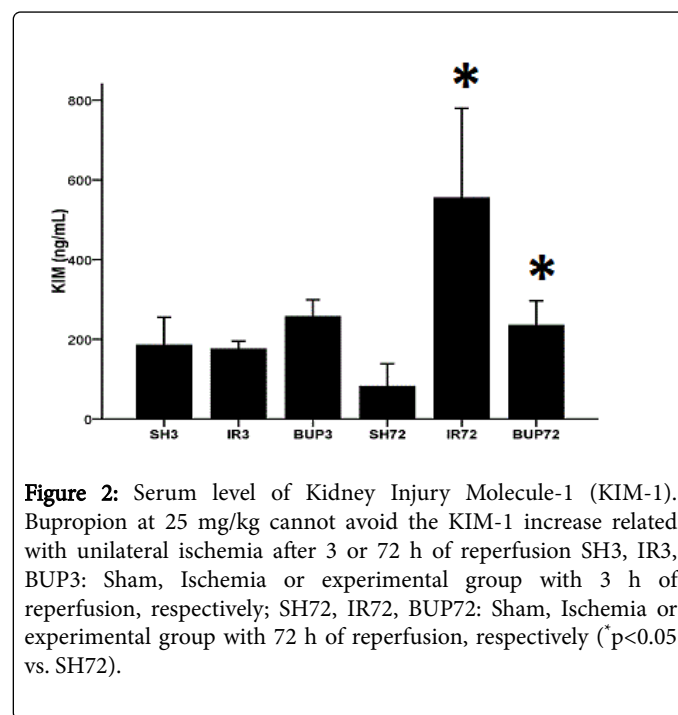

Figure 2: Serum level of Kidney Injury Molecule-1 (KIM-1). Bupropion at 25 mg/kg cannot avoid the KIM-1 increase related with unilateral ischemia after 3 or 72 h of reperfusion SH3, IR3, BUP3: Sham, Ischemia or experimental group with 3 h of reperfusion, respectively; SH72, IR72, BUP72: Sham, Ischemia or experimental group with 72 h of reperfusion, respectively (*$p<0.05$ vs. SH72).

Discussion

Ischemia can trigger an array of pathologic events that can activate injury mechanisms such as oxidative damage, apoptosis, nitric oxide synthase, excitotoxicity, transcription factors, growth factors, mitochondrial metabolism, angiogenesis, and inflammation [12,13]. Today it is possible to modify the course of an injury, through treatments applied before, during or following the damaging event, in the context of ischemia-reperfusion injury; preconditioning is understood as a therapeutic process aimed at improving tolerance of organs and tissues to potentially lethal ischemic injury; initially, it was described the ischemic preconditioning. However, the understanding of mechanisms that mediate ischemic preconditioning has allowed induction of a similar protection without ischemia: pharmacologic preconditioning [13,14].

Bupropion could reduce the inflammatory response [9] on pathologies such as Crohn's disease [15] and intestinal injury due to I/R [10], so we test it on renal I/R injury. Bupropion is a widely used as an antidepressant, smoking cessation aid, and weight-loss therapy [16] and is almost exclusively cleared by hepatic metabolism via CYP450 and its metabolites are excreted in the urine [17].

Bilateral renal pedicle clamping is commonly used to induce AKI, to improve post-injury survival and obtain more consistent and predictable results can be used models of unilateral I/R injury followed by contralateral nephrectomy [18]. Initially this model was used to evaluate the Bupropion on renal damage but the mortality was absolute at 48 h. There is evidence that the deterioration of renal function can alter the hepatic metabolism of some drugs [17]. In this context the drug pharmacokinetics may be altered by renal failure that decreases the excretion of actives metabolites, and increases in the accumulation of the drug and its metabolites by alteration of hepatic metabolism [6]. On monorrenal animals we could corroborate the injury secondary to ischemia, but in at least, 24 h of reperfusion, was not possible to identify any beneficial effect of bupropion on the AKI; DeVane and cols report the accumulation of some bupropion metabolites in guinea pigs with renal failure [19], and there is evidence that on patients with impairment of renal function, the accumulation of bupropion metabolites maybe reach toxic levels [20] Recently, the ERBP (European Renal Best Practice recommended a dose reduction of some antidepressant, included bupropion, in patients whit chronic kidney disease [21]. In our experiments, it was necessary to preserve renal clearance to avoid Bupropion toxicity. All the rats that kept their right kidney survived left kidney I/R injury.

AKI can be encompassing various etiologies; a prerenal injury like I/R triggers an intrinsic failure involving glomerular, tubular and/or interstitial damage; and after, the obstruction of tubular drainage as post-renal injury complicates the evolution. In non-nephrectomized rats with unilateral ischemia, Bupropion could not stop the histological damage in the I/R kidney (Figure 1).

NGAL and KIM-1 are biomarkers to monitor tubular injury, treatment with Bupropion cannot avoid the increase in AKI biomarkers (KIM-1) (Figure 2 and Table 2); Particularly KIM-1 and NGAL can be detected at 2 h of reperfusion. [22,23]. Bupropion does not decrease significantly TNF-α levels (p=0.06) despite identifying a tendency of lowering, but it was necessary to conserve the renal function of the right kidney (Table 2). Elsewhere, the inflammation process plays an important role in AKI; however, a decrease in inflammation may not be enough to improve its evolution.

Conclusion

Pharmacologic preconditioning with bupropion decreases TNF-α, but does not improve the evolution of AKI in rats undergoing renal I/R injury.

Acknowledgment

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