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Nephroprotective effects of (−)-α-bisabolol against ischemic-reperfusion acute kidney injury

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ABSTRACT

Background: Ischemia/reperfusion (I/R) in kidney is commonly related to acute kidney injury (AKI), essentially through oxidative stress. (−)-α-Bisabolol is a sesquiterpene isolated from the essential oil of a variety of plants, including chamomile, which has important antioxidant activity.
Study design: This study intends to evaluate the nephroprotective activity of (−)-α-bisabolol (Bis) in both *in vivo* and *in vitro* models of kidney I/R.
Methods: Male Wistar rats were submitted to right nephrectomy, followed by ischemia by clamping of the renal artery in the left kidney for 60 min. and 48 h of reperfusion. The animals were treated orally with Bis (100 mg/kg) or vehicle for 24 h after reperfusion, and placed in metabolic cages, to evaluate water consumption, diuresis, urinary osmolality, classic biochemical markers and urinary KIM-1 (kidney injury molecule-1). Additionally, the left kidney was collected for histological evaluation and determination of glutathione (GSH) and Thiobarbituric Acid Reactive Substances (TBARS) levels. Tubular epithelial cells LLC-MK2 were used to assess Bis effect on *in vitro* I/R, by MTT assay. It was performed the cellular respiration tests by flow cytometry: evaluation of the production of cytoplasmic reactive oxygen species by DCFH-DA assay and mitochondrial transmembrane potential analysis with the dye rhodamine 123.
Results: I/R caused alterations in diuresis, water intake, urinary osmolality, plasmatic creatinine, urea and uric acid, creatinine clearance, proteinuria and microalbuminuria. Treatment with Bis ameliorated all of these parameters. Also, KIM-1 level enhanced by I/R was also diminished in groups treated with Bis. The histological examination showed that Bis attenuated the morphological changes caused by I/R, markedly vascular congestion and intratubular deposits of proteinaceous material. Additionally, Bis was able to reduce the changes observed in TBARS and GSH levels in kidney tissue. In *in vitro* assay, Bis was capable to partially protect the cell lineage against cell damage induced by I/R.
Conclusion: (−)-α-Bisabolol has a nephroprotective effect in kidney I/R, with antioxidant effect. Moreover, this result seems to be associated to a direct protective effect on tubular epithelia.

Abbreviations: I/R, ischemia/reperfusion; AKI, acute kidney injury; Bis, (−)-α-bisabolol; KIM-1, Kidney Injury Molecule-1; ROS, reactive oxygen species; ELISA, Enzyme Linked Immunosorbent Assay; MDA, malondialdehyde; TBARS, Thiobarbituric Acid Reactive Substances; GSH, reduced glutathione; DTNB, 5,5′-Dithiobis-(2-nitrobenzoic acid); DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; Uosm, urinary osmolality; CrCl, creatinine clearance; GFR, Glomerular filtration rate; $FeNa^+$, fractional excretion of sodium; FeK^+, fractional excretion of potassium; $FeCl^-$, fractional excretion of chloride; uKIM-1, Urinary Kidney Injury Molecule-1; GSSG, Oxidized glutathione; GR, GSSG reductase enzyme; DCFH-DA, dichlorofluorescein diacetate; Rho123, rhodamine 123.

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Introduction

Acute kidney injury (AKI) may be defined as a sudden and unexpected loss or reduction of renal function, resulting in the accumulation of nitrogenous substances (urea and creatinine), with or without changes in diuresis (Li et al., 2013). AKI is associated with high morbidity and mortality and is part of the multiple organ dysfunction syndrome (Horkan et al., 2015). Studies indicate that AKI affects approximately 20% of hospitalized patients and up to 67% of those admitted to an intensive care unit, making it one of the most common organ dysfunctions (Harris et al., 2015). Also, the ischemia/reperfusion process is commonly related to AKI, primarily in renal transplanted patients (Ditonno et al., 2013).

The reperfusion injury occurs essentially through oxidative stress (Bussmann et al., 2014). The degradation of adenosine triphosphate (ATP) to adenosine, inosine, hypoxanthine and xanthine is possibly related with the damage caused by the ischemia and reperfusion events. At the stage of reperfusion of the ischemic tissue, the accumulation of xanthine results in the production of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) (Shimo et al., 2011). The damage to the renal tubule cells caused by ROS is mediated by protein oxidation, lipid peroxidation, DNA damage and cell death induction. Peroxidation of membrane phospholipids results in increased cell permeability and changes in renal function and histology (Devarajan 2006).

Noteworthy, biomarkers are indeed useful and should be incorporated as endpoints in experimental AKI studies, in particular for tracing early and subtle damage, Therefore, the Kidney Injury Molecule-1 (KIM-1) is a novel biomarker for renal proximal tubule injury, undetectable in normal kidneys, but is markedly elevated in acute renal injury (Ichimura et al., 2008). It is considered a unique marker with high sensitivity and specificity but, above all, used for the diagnosis of early kidney damage (Tan et al., 2015).

(−)-α-Bisabolol is a small oily sesquiterpene alcohol with a molecular mass of 222.37 g/mol ($C_{15}H_{26}O$) isolated from the essential oil of a variety of plants, shrubs and trees. It was first isolated in 1951 by Isaac (Brunke and Hammaerschmidt 1985) from the essential oil of *Matricaria chamomilla* (*Asteraceae*), popularly known as chamomile, which contains up to 50% of α-bisabolol (Kamatouand and Viljoen 2010; Jakovlev and Von Schlichtegroll 1969). Generally, (−)-α-Bisabolol can be obtained by hydrodistillation of the respective essential oils. Examples of these plants are German chamomile (*M. chamomilla*), sage (*Salvia runcinata*), *Vanillosmopsis* sp. and *M. crassifolium* (Buitrago et al., 2015).

There have been studies reporting that (−)-α-bisabolol has anti-infective (Rottini et al., 2015), cytotoxic (Liang et al., 2014), anti-inflammatory (Zargaran et al., 2014) and anticholinesterasic (de Siqueira et al., 2012) activity and promotes the transdermal permeation of drugs (Kim et al., 2012). However, most of the known biological effects of (−)-α-Bisabolol are due to its antioxidant activity (Agatanovic-Kustrin et al. 2015).

(−)-α-Bisabolol becomes of paramount importance when studying this injury, which basically has an oxidative physiopathology. This terpene has a well described antioxidant activity. Moreover, it is also important to study AKI onset through the biochemical alterations related to the damage caused by the ischemia and reperfusion injury, as well as the involvement of KIM-1 in the early diagnosis.

Thus, the present study intends to evaluate the bioprospecting of (−)-α-bisabolol as a molecule with nephroprotective activity against the injury caused by the ischemia followed by reperfusion (I/R) process.

Methods

Animals and (−)-α-bisabolol

The experimental protocol was approved by the Ethical Committee on Animal Research of Universidade Federal do Ceará (UFC) (no. 142/2015) in accordance with the ethical guidelines. Male Wistar rats, obtained in the central bioterium of UFC, weighting approximately 200 g, were maintained under controlled conditions (25 ± 2 °C ambient temperature, 12 h light-dark cycle). Food and water were offered *ad libitum*. (−)-α-bisabolol was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Surgical procedure

Animals were anesthetized with Thiopental (Cristália Pharmaceutical and Chemical, São Paulo, Brazil) (50 mg/kg i.p.). A midline laparotomy incision was performed, the right kidney was removed and the ischemia was induced in the left kidney, as described on graphical abstract, by non-traumatic clamping of the renal artery for 60 min, followed by 48 h of reperfusion as previously described (Tillet et al., 2015; Tucci et al., 2008).

During surgery, the animals were kept warm in thermal blanket in the range of 36.5–37 °C. To minimize dehydration, the abdominal area was covered with saline-soaked gauze. After the procedure, temperature, heart rate and respiratory frequency were checked every half hour. Surgery will not be started until the body temperature is stabilized at the set-point, and the mouse is in deep anesthesia and thus does not respond to pain induced by toe pinch.

Sham operations were performed in a similar surgical procedure, except for the clamping of renal artery. During the last 24 h, urine samples were obtained through a metabolic cage. At the end of this period, animals were reanesthetized to obtain blood samples for biochemical tests. Additionally, the left kidneys were collected for histological evaluation. Euthanasia was performed on anesthetized animals by administration of saturated solution of potassium chloride intravenously, as described by the ethical guidelines.

Experimental groups

The animals were divided into 4 experimental groups ($n = 6$), all of these treated through oral gavage 24 h after reperfusion onset: Sham, treated with vehicle; Sham + Bis, treated with (−)-α-bisabolol (100 mg/kg); ischemia/reperfusion (I/R), surgical group treated with vehicle; I/R + Bis, surgical group treated with (−)-α-bisabolol (100 mg/kg). Bisabolol was diluted in DMSO 2% and the dose was used as previously described (Bezerra et al., 2009).

Measurement of biochemical parameters

Blood samples were collected in tubes containing lithic heparin, and then centrifuged (4500 rpm for 10 min) to collect plasma. Proteinuria, microalbuminuria, plasmatic and urinary levels of creatinine and plasmatic levels of urea and uric acid were measured using an automatic analyzer (Roche Diagnostics Limited, Rotkreuz Switzerland) and plasmatic and urinary levels of sodium (Na^+), potassium (K^+) and chloride (Cl^-) were measured using an ion selective electrode (Roche Diagnostics Limited, Rotkreuz Switzerland). creatinine clearance (CrCl) was calculated using the formula: $CrCl = (V_{min} \times uCr)/sCr$, where V_{min} is urinary volume per minute, uCr and sCr are, respectively, urinary and serum creatinine (Darling and Morris, 1991). Additionally, water consumption and diuresis were recorded and urinary osmolality was measured with a freezing-point depression osmometer (PZL, Londrina, Brazil).