



Mesenchymal stem cell therapy promotes the improvement and recovery of renal function in a preclinical model

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Abstract

Acute renal failure (ARF) is an extremely important public health issue in need of novel therapies. The present study aimed to evaluate the capacity of mesenchymal stem cell (MSC) therapy to promote the improvement and recovery of renal function in a preclinical model. Wistar rats were used as the experimental model, and our results show that cisplatin (5mg/kg) can efficiently induce ARF, as measured by changes in biochemical (urea and creatinine) and histological parameters. MSC therapy performed 24h after the administration of chemotherapy resulted in normalized plasma urea and creatinine levels 30 and 45d after the onset of kidney disease. Furthermore, MSC therapy significantly reduced histological changes (intratubular cast formation in protein overload nephropathy and tubular hydropic degeneration) in this ARF model. Thus, considering that current therapies for ARF are merely palliative and that MSC therapy can promote the improvement and recovery of renal function in this model system, we suggest that innovative/alternative therapies involving MSCs should be considered for clinical studies in humans to treat ARF.

Keywords: bone marrow, chemotherapy, nephrotoxicity, kidney disease, tissue regeneration.

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Introduction

Acute renal failure (ARF) is characterized by the accumulation of nitrogen-containing compounds (urea and creatinine) in the blood (with or without oliguria) followed by the loss of renal function, ultimately blocking the excretion of waste nitrogen and maintenance of homeostasis (Thadhani *et al.*, 1996).

ARF is associated with high mortality and morbidity rates and is reported in approximately 4-5% of all hospitalization cases. This disease can develop into more severe forms, resulting in death in approximately 50-70% of pa-

tients, making ARF an extremely important public health issue (Hoste and Schurgers, 2008; Wen *et al.*, 2010).

ARF can be caused by a number of factors, including decreased renal perfusion without cellular injury, an ischemic, toxic or obstructive insult to the renal tubules, a tubule-interstitial process with inflammation and edema, or a primary reduction of glomerular filtering capacity, among others (Thadhani *et al.*, 1996). Chemo- and radiotherapy are also important causes of ARF (Humphreys *et al.*, 2005). Among chemotherapeutic drugs, cisplatin, which is used to treat the majority of solid and hematological tumors (Serpeloni *et al.*, 2013), is most associated with nephrotoxicity due to its high concentrations in the kidneys, even at non-toxic plasma levels, as well as its adverse impact on the renal transport system (Gordon and Gattone 1986; Ren *et al.*, 2002; Serpeloni *et al.*, 2013).

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Generally, ARF is incurable, and current therapeutic strategies involve simply removing or treating its cause (Fry and Farrington, 2006). As ARF is prone to complications and chronicity, innovative/alternative therapies for the quick, safe and efficient recovery of renal function are increasingly required.

Currently, models of ARF involving cell therapy have been effective and promising (Yokoo *et al.*, 2003; Brodie and Humes, 2005; Zerbini *et al.*, 2006; Choi *et al.*, 2010; Papazova *et al.*, 2015). In the present study, we investigated the capacity of mesenchymal stem cell (MSC) therapy to promote the improvement and recovery of renal function in a pre-clinical model.

Material and Methods

Experiment 1

Chemical agents

ARF was induced by means of a single-dose administration of the chemotherapeutic agent cisplatin (Fauldcispla LIBBS®, Brazil, Lot: 12GO702) at 5mg/kg body weight (bw) via intraperitoneal (ip) injection.

Animals

Fifty-nine sexually mature male Wistar rats were obtained from the Central Animal House of the Center of Biological and Health Sciences of the Federal University of Mato Grosso do Sul (Centro de Ciências Biológicas e da Saúde da Universidade Federal de Mato Grosso do Sul – CCBS/UFMS). The animals were housed in groups of three in polypropylene cages containing wood shavings as bedding and were fed a commercial diet (Nuvital®, Brazil) and filtered water *ad libitum*. The cages were kept in a ventilated cabinet (Alesco®, Brazil) under standard climate-controlled conditions with a 12-h photoperiod (12:12 h LD), a temperature of 22.2 °C, and a relative humidity of 55.10%. The experiment was conducted in accordance with the guidelines of the Universal Declaration of Animal Rights and was approved by the Animal Research Ethics Committee of the UFMS (Protocol #499/2012).

Experimental design

Initially, the animals were divided into two lots. Lot I consisted of 10 animals, which were used as bone marrow-derived MSC donors. Lot II consisted of 49 animals, which were further subdivided into three experimental groups. Control Group (CG; $n = 14$) animals were treated with a single dose of 1 mL/100g (bw; ip) phosphate-buffered saline (PBS), followed by an intravenous (iv) dose of 1 mL PBS via tail vein injection after 24 h. Control animals were evaluated at 30 days (CG-30d; $n = 7$) and 45 days (CG-45d; $n = 7$) after the onset of treatment. The ARF Group (ARFG; $n = 21$) animals were treated with a single dose of cisplatin at 5 mg/kg (bw; ip), followed by 1 mL PBS (iv) 24-h later. ARF animals were then evaluated at 48 h

(ARFG-48h; $n = 7$), 30 days (ARFG-30d; $n = 7$), and 45 days (ARFG-45d; $n = 7$) after cisplatin treatment. The ARFG + MSC Group (ARFG+MSC; $n = 14$) animals were treated with a single dose of cisplatin at 5mg/kg (bw; ip), followed by treatment with MSCs in 1 mL PBS (iv) 24h later. Animals were then evaluated at 30days (ARFG+MSC-30d; $n = 7$) and 45days (ARFG+MSC-45d; $n = 7$) after cisplatin treatment.

Assessment of renal function and confirmation of acute renal failure

ARF was confirmed indirectly by measuring serum urea and creatinine levels. Peripheral blood was collected under anesthesia (ketamine – 50 mg/kg bw, ip; xylazine – 10mg/kg bw, ip) from the retro-orbital plexus, either at 48h in the ARFG group (used as the standard for the establishment of ARF), or at 30d and 45d after cisplatin administration in the other groups. The first blood sample was drawn from animals in the ARFG-48h group 7d prior to the administration of cisplatin, which was used as an internal control for the experiment. After collection, the blood was allowed to sediment at 4 °C, and the serum was then stored at -20 °C until the time of analysis. Analyses were performed using an automated COBAS 6000 analyzer (Roche Diagnostics®, Brazil), according to the manufacturer's recommendations.

Isolation and expansion of bone marrow-derived mesenchymal stem cells

Donor animals from Lot I were euthanized by anesthetic overdose (Thiopentax® Cristalia Laboratories, Brazil, Lot: 12075226). Femurs were then collected under sterile conditions, and the bone marrow was flushed with 5mL PBS supplemented with 1% antibiotics (penicillin/streptomycin, LGB Biotecnologia®, Brazil). Cell suspensions were centrifuged for 5 min at 670 x g, and the pellet was resuspended with 5 mL sterile PBS; resuspension was performed three times. The pellet was then resuspended with 4 mL Dulbecco's Low Glucose Modified Eagle's Medium (DMEM-low glucose; LGC Biotecnologia®, Brazil), transferred to a conical tube containing 4 mL SeptCell (LGC Biotecnologia®, Brazil), and then centrifuged for 30 min at 241 x g. The mononuclear cell fraction was collected and resuspended with 5 mL PBS, centrifuged for 5 min at 670 x g, and then resuspended with PBS. This last step was repeated five times before the pellet from each donor animal was seeded into two 25-cm² culture flasks containing 10 mL of DMEM-low glucose supplemented with 10% Fetal Calf Serum (LGC Biotecnologia®, Brazil) and 1% antibiotics (penicillin/streptomycin, LGB Biotecnologia, Brazil). Cell viability was assessed by adding an equal volume of trypan blue (LGC Biotecnologia®, Brazil) to 10 µL samples from each donor.

Cells were cultured in a CO₂ incubator (Thermo Scientific®, USA) at 37 °C under 5% CO₂. During expansion, the supplemented culture medium was replaced every 48 h.

During each passage (cultures at 70–80% confluence), the cells were washed three times with 5 mL PBS and then trypsinized by adding 1 or 2 mL of 0.25% trypsin (LGC Biotecnologia®, Brazil) to the 25- or 75-cm² flasks, respectively. The trypsin was inactivated by adding 5 volumes of supplemented culture medium. The cell suspension was then centrifuged for 5 min at 670 x g, and the pellet was resuspended with 1 mL of supplemented culture medium. For the first passage, each 25-cm² flask was divided into two 25-cm² flasks. For the second and third passages, cells were seeded into 75-cm² flasks at 1:1 and 1:2 ratios, respectively. During each passage, 10 µL samples were collected for trypan blue analysis of cell viability.

Osteogenic, adipogenic and chondrogenic differentiation

Flasks intended for differentiation experiments were trypsinized as described above after reaching 80% confluence; four 25-cm² flasks were seeded with 2 × 10⁵ cells each, and two 15-mL conical tubes were seeded with 1 × 10⁶ cells each. Two 25-cm² flasks and one conical tube were used as controls and cultured with supplemented culture medium. Cells used for osteogenic and adipogenic differentiation were preincubated with supplemented culture medium for 24 h and then cultured for 14 d with culture medium from the STEMPRO Osteogenesis or Adipogenesis Differentiation Kit (Invitrogen®, USA), respectively. For chondrogenic differentiation, cells were pre-incubated in supplemented medium for 2 h and then cultured for 21 d with culture medium from the STEMPRO Chondrogenesis Differentiation Kit (Invitrogen®, USA). Culture media from all of the above differentiation cultures were changed twice weekly. At the end of the respective culture periods, media were removed and cells were fixed in paraformaldehyde for 1 h. Adipogenic and osteogenic differentiation were assessed by staining with Oil Red O and Alizarin Red S, respectively. Chondrogenic differentiation was assessed by submitting the pellet to conventional histological processing and staining with toluidine blue (Hermeto *et al.*, 2015).

Mesenchymal stem cell transplantation

After reaching confluence during the third passage, 49 culture flasks originated from the first unique 10 donors were trypsinized and randomly transplanted to the 49 receptor animals from the different experimental groups. Suspended cells were centrifuged for 5 min at 670 x g, and the pellet was resuspended with PBS to obtain 1.0 × 10⁶ cells for use in cell therapy. Animals from the ARFG+MSC received MSC transplant (iv) 24 h after cisplatin administration, whereas animals from the remaining groups were treated with 1 mL of MSC suspension vehicle (sterile PBS; iv). For transplantation, the animals were anesthetized as described above.

Histopathological analysis

At the end of each experimental period (48 h, 30 d and 45 d), the animals were euthanized by an anesthetic over-

dose, as described above. Kidneys were collected, sectioned, fixed in 10% buffered formaldehyde and prepared according to routine histopathological practices. Briefly, tissue fragments were dehydrated after fixation, cleaned, and then embedded in paraffin. Samples were prepared using a microtome (4-µm thick slices) and stained with hematoxylin/eosin (HE) for histopathological analysis by bright-field microscopy (1000x magnification).

Slides were submitted to double-blind histopathological analysis according to the Banff 97 Classification Criteria: 1) tubulitis: intratubular lymphocyte infiltration (necrosis and the degree of fibrosis were not evaluated); 2) tubular hydropic degeneration: cytoplasmic ballooning of cells in the proximal convoluted tubule (1+ < 25%; 2+ 25–75%; 3+ > 75%); 3) intratubular cast formation: eosinophilic deposits within tubules (proximal convoluted and distal tubule, Henle's loop, failure of glomerular filtration, failure of tubule reabsorption) (1+ < 25%; 2+ 25–75%; 3+ > 75%); 4) glomerulitis: lymphocyte infiltration in the glomeruli; 5) arteritis: lymphocyte infiltration in the arterioles; 6) interstitial infiltration: lymphocytes in the interstitial space (1+ 0–25%; 2+ 25–75%; 3+ > 75%); 7) interstitial fibrosis: scar collagen deposition; 8) glomerulosclerosis: fibrosis in the glomeruli (nephron failure); 9) calcifications; 10) cortical retraction/atrophy: partial substitution of kidney parenchyma by fibrosis and/or inflammatory infiltration; 11) tubular apoptosis/necrosis: total or partial tubule degeneration (1+ < 25%; 2+ 25–75%; 3+ > 75%; and 12) global necrosis: involvement of the glomeruli and tubules (Racusen *et al.*, 1999).

Statistical Analysis

Statistical analysis was performed using the SigmaPlot statistics software program (Version 12.5), with the significance level set at 5%. The applied non-parametric tests included the Mann-Whitney *U* test for comparisons between two experimental groups, and the Kruskal-Wallis test, followed by Dunn's test, for comparisons between more than two experimental groups.

Experiment 2: Mesenchymal stem cell localization and migration

Experimental animals, design and conditions

Six sexually mature male Wistar rats were divided into two experimental groups (*n* = 4): ARFG and ARFG+MSC. Animals were evaluated 48 h after the transplantation of MSCs that were previously stained with the nuclear marker 4',6-diamidino-2-phenylindole (DAPI, Life Technologies®). Animal housing conditions, determination of renal function, confirmation of ARF, anesthesia, and euthanasia, as well as isolation, expansion and transplantation of MSCs were performed as described for Experiment 1. However, prior to transplantation, MSCs were incubated with 10 µg/mL of DAPI in the dark for 30 min at 37 °C.

Histological fluorescence analysis

After euthanasia, animal kidneys were collected and immediately frozen in liquid nitrogen. Organs were then mounted on a holder with cryostat embedding medium (EasyPath), and 5- μm thick slices were cut at -10°C in the dark. Sections were placed on slides and analyzed by fluorescence microscopy (BA410 FL) with a DAPI filter (EX D350/50x; DM 400DCLP; BA D460/50m) at 400x magnification.

Results

Expansion of mesenchymal stem cells

After the isolation procedure, MSCs were subjected to an expansion protocol with consecutive passages. For all passages, only cultures with $> 95\%$ viability were maintained.

Mesenchymal stem cells differentiate into osteogenic, adipogenic and chondrogenic cells in vitro

Bone-marrow-derived MSCs obtained from donor rats were induced to differentiate, and characteristic morphological changes were observed in these cell types. Figure 1 shows representative images of undifferentiated control cells cultured on an adherent surface (Control), as well as adipogenic and osteogenic differentiation (Differentiation). Osteogenesis was assessed by means of Alizarin Red S staining, which labels calcium deposits in differentiated cells, and adipogenesis was assessed by means of Oil Red O staining, which labels fat vesicles and vacuoles in adipogenic cells. The lower row shows undifferentiated cells from pellet cultures (Control), and chondrogenic differentiated MSCs, the latter showing toluidine blue staining, indicating increased extracellular matrix deposition by differentiated chondrocytes.

Mesenchymal stem cell localization and migration

DAPI-stained MSCs migrated from the bloodstream to the kidneys, and their localization was confirmed by fluorescence microscopy. Figure 2 shows a stained MSC suspension (A), a renal cortex with no evidence of MSC infiltration (B), and a renal cortex exhibiting MSCs stained with DAPI (C).

Validation of the cisplatin-induced acute renal failure model

Plasma creatinine and urea levels were measured in the ARFG-48h group 7 d before and 48 h after the administration of cisplatin, and statistically significant increases in the levels of these biomarkers confirmed the onset of ARF (Figure 3A,B). Mean urea and creatinine levels were 43.43 ± 4.07 and 0.40 ± 0.00 , respectively; after cisplatin admin-

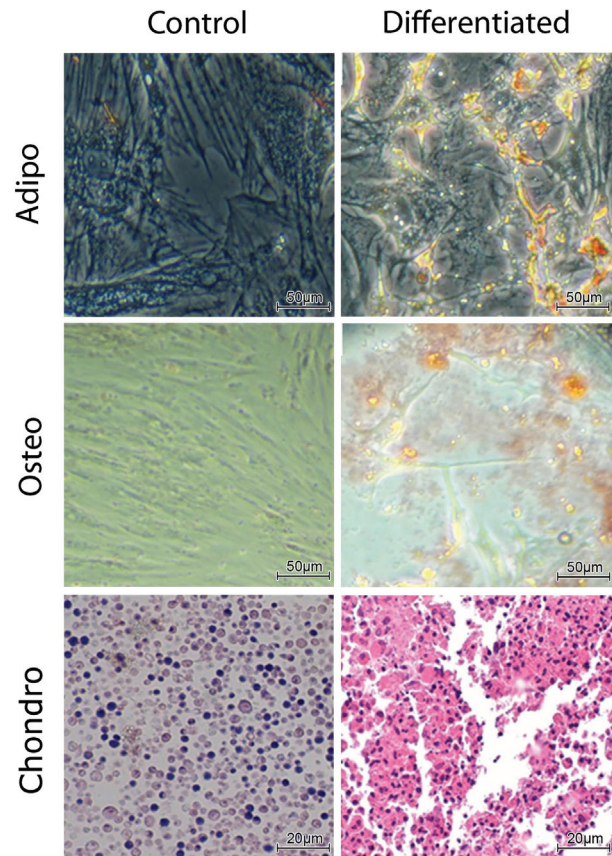


Figure 1 - MSC are able to differentiate into adipogenic, osteogenic (scale bars: 50 μm) and chondrogenic (scale bars: 20 μm) lineages, as shown by the staining.

istration, levels increased ($p \leq 0.05$) to 218.00 ± 64.42 and 1.89 ± 0.26 , respectively.

Based on urea and creatinine levels 30 d after the administration of chemotherapy in the ARFG group, we concluded that cisplatin treatment successfully induced ARF, which persisted throughout the experimental period, exhibiting mean values of 213.29 ± 63.23 and 1.94 ± 0.40 , respectively. By contrast, in the ARFG+MSC, transplantation of MSCs 24h after chemotherapy led to improved renal function, with mean urea and creatinine values of 59.14 ± 4.53 and 0.53 ± 0.05 , respectively. Importantly, these values were statistically similar ($p > 0.05$) to those from the CG group (47.43 ± 1.54 and 0.40 ± 0.00 for urea and creatinine, respectively).

At 45 d, creatinine levels were no longer significantly different ($p > 0.05$) between experimental groups, whereas urea levels remained high ($p \leq 0.05$) in the ARFG group (90.5 ± 10.56) compared with the CG (44.14 ± 4.36) and ARFG+MSC (52.57 ± 1.19) groups; the latter two groups were not significantly different from one another ($p > 0.05$).

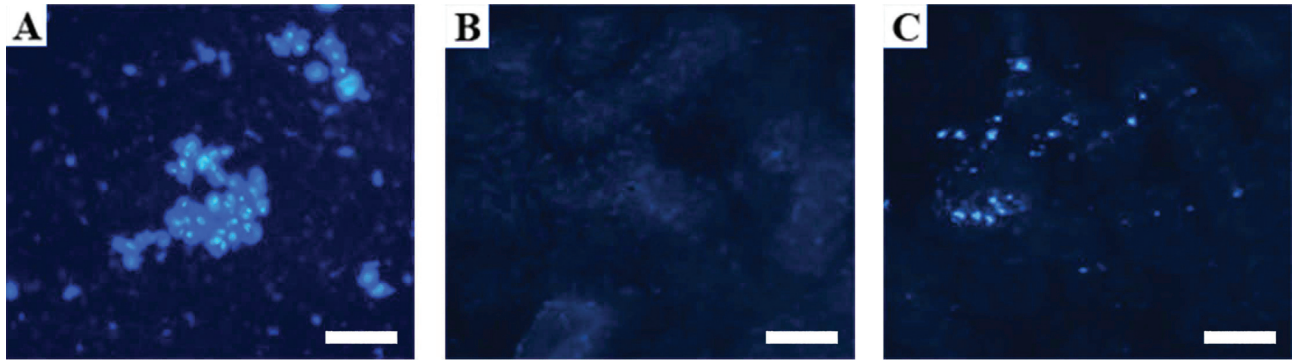


Figure 2 - DAPI staining: (A) MSC stained with DAPI; (B) renal cortex without MSC infiltration; (C) renal cortex with MSC infiltration, 48 h after the transplant. Scale bars: 20 μ m.

Histopathological analysis

Histopathological analysis of the ARFG-48h group revealed tubular hydropic degeneration, intratubular cast formation, tubular necrosis/apoptosis, and global necrosis, with medians of 3, 3, 2 and 1, respectively.

In the ARFG and ARFG+MSC groups, the observed changes were tubulitis, tubular hydropic degeneration, intratubular cast formation, interstitial infiltration, interstitial fibrosis, and cortical atrophy – irrespective of the period of analysis (30 d or 45 d) (Figure 4A, Figure 5).

Comparisons between the ARFG-30d and the ARFG+MSC-30d groups revealed a statistically significant reduction in intratubular cast formation, with medians of 1 and 0, respectively. Conversely, there was no statistically significant difference between the ARFG-30d and the ARFG+MSC-30d groups with respect to tubulitis, tubular hydropic degeneration, interstitial infiltration, interstitial fibrosis, or cortical atrophy (medians of 0, 1, 1, 0 and 0 vs. 1, 1, 1, 0 and 1, respectively) (Figure 4B).

When comparing the ARFG-45d and the ARFG+MSC-45d groups, there was a statistically significant reduction in tubular hydropic degeneration (medians of 1 and 0, respectively) and intratubular cast formation (medians of 1 and 0, respectively). No significant differences were observed between the ARFG-45d and ARFG+MSC-45d groups with respect to tubulitis, interstitial infiltration, interstitial fibrosis, or cortical atrophy (medians of 0, 1, 1, 0 vs. 0, 0, 0, 0, respectively) (Figure 4C).

Discussion

Preclinical models are currently being used to assess the effects of MSCs on various nephropathies. Of the several types of kidney disease that affect humans, renal failure is the primary target of modern bioengineering approaches, as cell therapy can replace specific cells types that have been lost in renal patients (Brodie and Humes, 2005). Such studies have addressed how cell therapy can help regenerate convoluted tubules and promote the normalization of biochemical parameters after an ischemic or toxic insult to

the kidney (Lin *et al.*, 2003; Lin, 2006). However, the mechanisms involved in these processes remain poorly understood, and further studies are needed to extend these findings.

The MSCs used in the present study were characterized by adipogenic, osteogenic and chondrogenic differentiation. These results are consistent with previous studies (Bagnaninchi *et al.*, 2011; Pendleton *et al.*, 2013; Vieira *et al.*, 2014; Hermeto *et al.*, 2015), as was the efficiency of the selection and expansion methods used here.

The migration of MSCs to the kidney and the permanence of these cells remain controversial topics. However, in the present study, MSCs were stained with DAPI prior to intravenous transplantation, and they were identified in the kidney 48h after cell therapy. This result is consistent with other studies showing the presence of MSCs in the kidney (Ross *et al.*, 2012; Tang *et al.*, 2012; Pan *et al.*, 2014). In addition to nuclear staining with DAPI, other protocols have also been used to detect MSCs in the kidneys, including tagging with green fluorescent protein (Qiu *et al.*, 2014), detection of β -galactosidase (β -Gal) (Klinkhammer *et al.*, 2014), and transplantation of cells derived from different sexes (*e.g.*, male donor and female recipient) (Grimm *et al.*, 2001). Despite these studies, some authors have reported an absence of MSCs in the kidney in experiments performed with MSCs and ARF (Duffield *et al.*, 2005).

These discrepancies aside, studies involving MSC transplants have yielded good results (Altun *et al.*, 2012; Liu *et al.*, 2012; Xinaris *et al.*, 2013; Erpicum *et al.*, 2014), and in general, exogenous MSC donor sources are used (Morigi *et al.*, 2004), as was the case in the present study.

Our results demonstrate that ARF is efficiently induced by treatment with cisplatin – a widely used cancer therapy drug whose major complication is nephrotoxicity (Nishihara *et al.*, 2013; Hagar *et al.*, 2015). Urea and creatinine levels were used as biomarkers to confirm ARF induction, and these were increased 48h after chemotherapy administration. ARF was also confirmed histologically by detecting tubular hydropic degeneration, intratubular

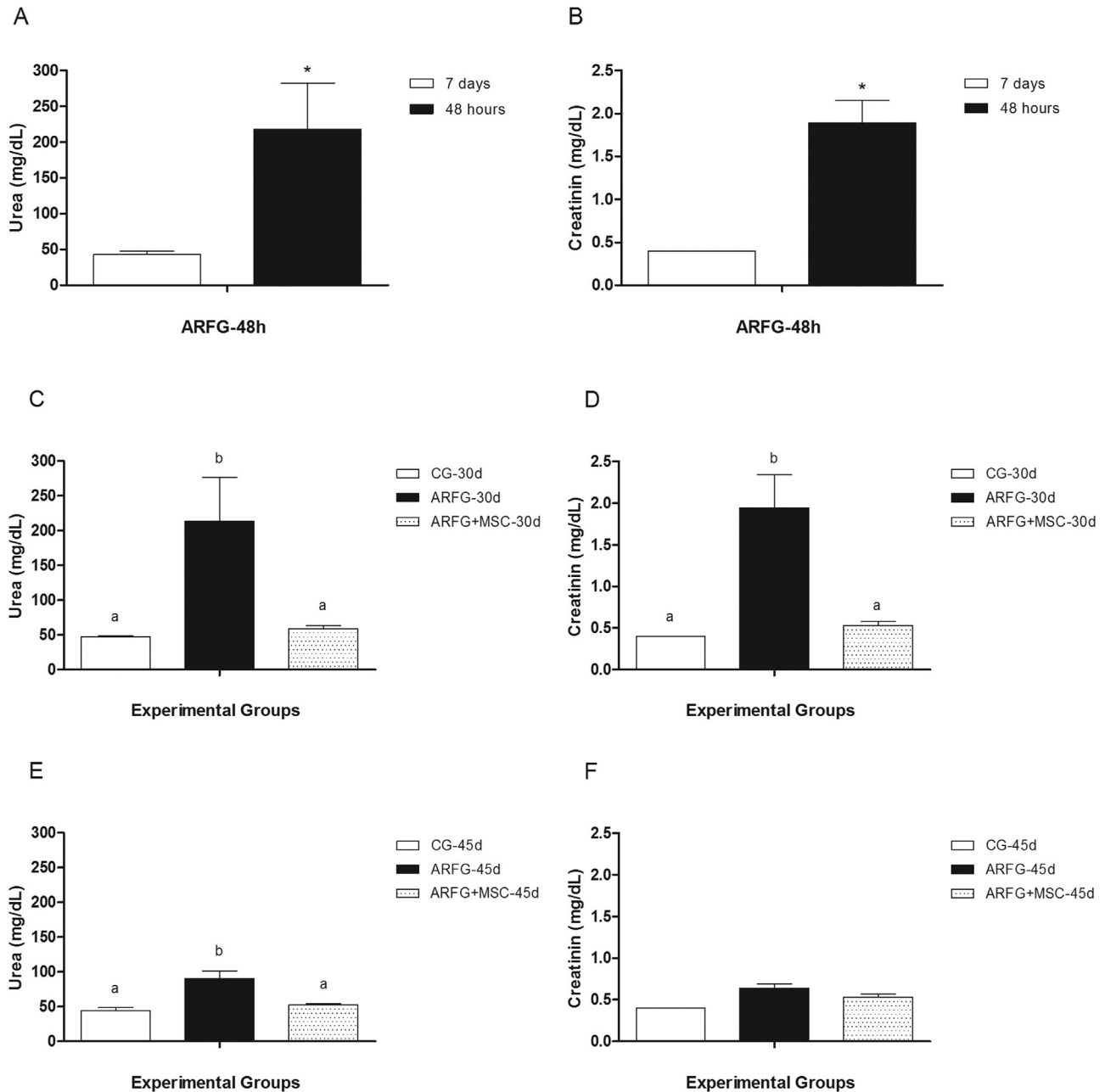


Figure 3 - Urea and creatinine plasmatic concentration (mean ± SD). A and B) Confirmation of acute renal failure. ARFG-48h: Acute renal failure group; animals were treated with cisplatin, evaluated seven days before and 48 h after the administration of the chemotherapy. C and D) Animals were evaluated 30 days after the administration of the chemotherapy. E and F) Animals were evaluated 45 days after the administration of the chemotherapy. CG: Control group. ARFG: Acute renal failure group; animals treated with cisplatin. ARFG+MSC: In this group, MSC were transplanted to animals with acute renal failure 24 h after the chemotherapy administration. Statistical analysis: A and B: Mann-Whitney, *statistically significant differences, $p \leq 0,05$; C to F: Kruskal-Wallis with Dunn's post-hoc test; different letters indicate statistically significant differences, $p \leq 0,05$.

cast formation, tubular necrosis and apoptosis, and global necrosis. We also observed that these biochemical and histological changes persisted 30d after cisplatin administration. At 45 d, only increased urea levels and histological damage could still be observed, whereas creatinine levels were back to normal (*i.e.*, not significantly different from the control group). These observations suggest that a single dose of 5 mg/kg (bw; ip) cisplatin in Wistar rats is sufficient

to efficiently induce ARF by toxic insult in this experiment model. Thus, this model can be used in studies to evaluate the events associated with patients under chemotherapy during the occurrence of ARF.

With respect to MSC transplantation, this method proved to be effective at improving the overall health of the animals undergoing cell therapy. Specifically, plasma urea and creatinine levels were efficiently reduced to levels sim-

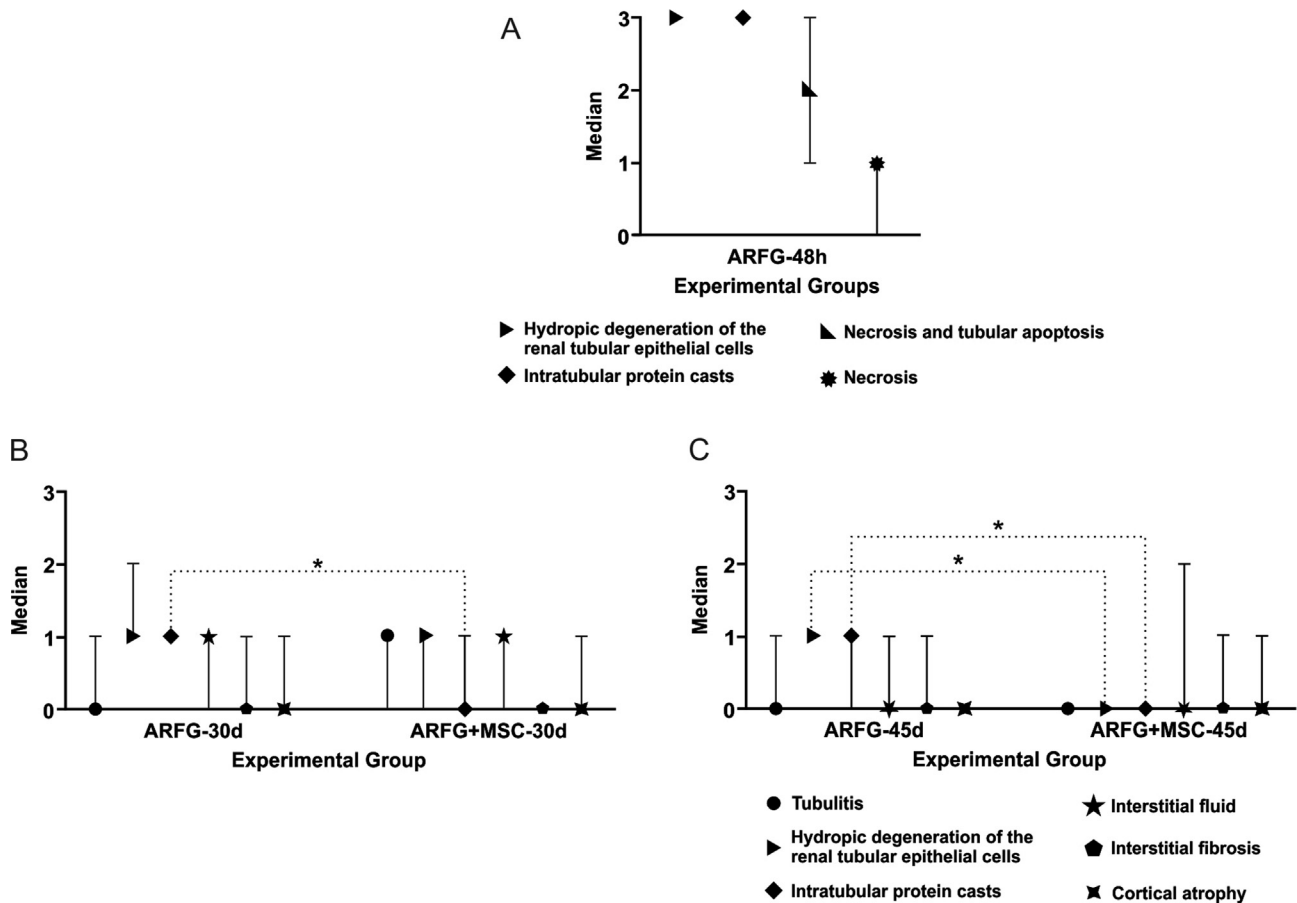


Figure 4 - Data from the histopathological cuts according the Banff97 classification. A) ARFG-48h: Acute renal failure group; animals were treated with cisplatin, evaluated seven days before and 48 h after the administration of the chemotherapy. B and C) ARFG: Acute renal failure group; animals treated with cisplatin. ARFG+MSC: In this group, MSC were transplanted to animals with acute renal failure 24 h after the chemotherapy administration. B) 30d – animals evaluated after 30 days of the administration of the chemotherapy. C) 45d – animals evaluated after 45 days of the administration of the chemotherapy. Statistical analysis: Mann-Whitney, *statistically significant differences, $p \leq 0.05$.

ilar to those found in the control group 30 d after the administration of chemotherapy. Additionally, histological analysis revealed significantly reduced intratubular cast formation over the same time period.

Forty-five days after cisplatin administration, the transplanted MSCs were still able to reduce plasma urea levels, yielding values similar to those in the control group, and histological analysis revealed statistically significant reductions in both tubular hydropic degeneration and intratubular cast formation.

The above results are consistent with other studies showing reductions in urea and creatinine levels in animals with ARF following cell therapy (Shih *et al.*, 2013; Geng *et al.*, 2014; Jiang *et al.*, 2015). Furthermore, in the studies by Imberti *et al.* (2007) and Qiu *et al.* (2014), improvements in histological parameters were also observed in animals with ARF who received MSC transplants.

Considering these results, we conclude that cell therapy with MSCs has therapeutic potential for ARF. Unlike some other organs, such as the heart or brain, the kidneys possess a high regenerative capacity following ischemic or

toxic insult, and tissue repair is thought to involve three phases: (I) inflammation – recruitment of immune cells; (II) regeneration – substitution of injured cells with new cells of the same type; and (III) repair – healing of interstitial tissues (Bonventre, 2003; Lin *et al.*, 2008). More specifically, tubules regenerate to recover renal function through elongation, mitosis and differentiation of the remaining uninjured cells, which ultimately line the denuded basement membrane (Thadhani *et al.*, 1996; Sheridan and Bonventre, 2000; Devarajan, 2006; Humphreys *et al.*, 2008).

With respect to the capacity of MSCs to promote renal tissue repair, these cells are thought to act by: (I) modulating the inflammatory response, which can induce phagocytosis in the spleen or liver to remove the MSCs from the bloodstream, where they are present for a short time (Duffield and Bonventre, 2005; Duffield *et al.*, 2005); (II) secreting growth factors; (III) merging with injured cells; or (IV) differentiating into new resident renal cells (Duffield and Bonventre, 2005; Tögel *et al.*, 2005; Humphreys and Bonventre, 2008).

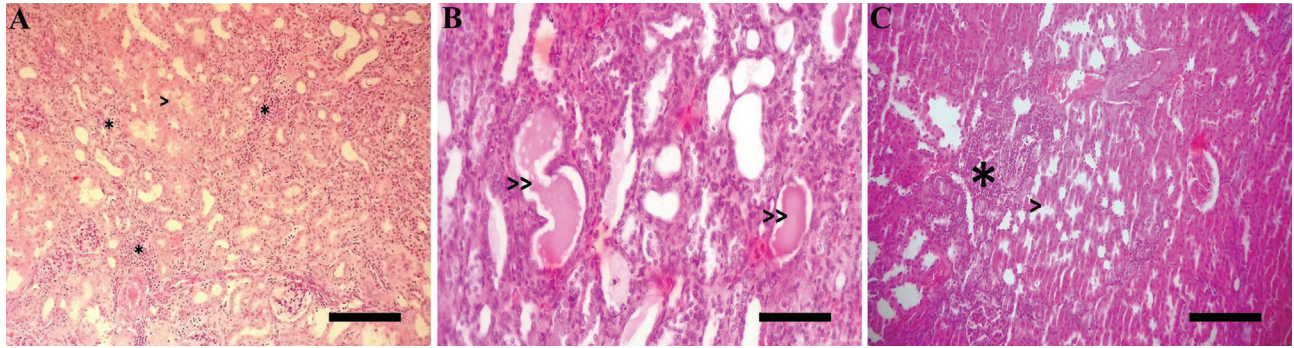


Figure 5 - Histological sections of the kidneys from cisplatin treated and MSC transplanted animals. A) Hydropic degeneration (>) and interstitial infiltration (*); scale bar: 100 μm . B) Intratubular cast formation (>>); scale bar: 25 μm . C) interstitial fibrosis (>) and interstitial infiltration (*); scale bar: 100 μm .

Our results support the notion that the effects of cell therapy are not based on repopulation and/or fusion of MSCs with the renal tubule (Duffield and Bonventre, 2005; Tögel *et al.*, 2005; Humphreys and Bonventre, 2008). However, in the present study, MSCs were found in the kidney 48h after cell therapy, and according to Morigi *et al.* (2006), Humphreys and Bonventre (2007, 2008), this time period is insufficient for the repopulation, fusion and/or transdifferentiation of MSCs towards reconstituting the renal epithelium. Also, the absence of MSCs on the renal tissue, observed by DAPI staining on days 5, 15, 30 and 45 (data not shown), corroborate these facts. An alternative explanation for the observed results is modulation at the paracrine level, considering that MSC therapy is related to increases in vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF) 1 (Lange *et al.*, 2005; Tögel *et al.*, 2005), as well as with reduced inflammation, through the induction of anti-inflammatory cytokines (Krampera *et al.*, 2006). Another possibility is that MSCs trigger renal stem cells to undergo mitosis, differentiate, and migrate, thus promoting renal tissue regeneration. Indeed, the latter hypothesis appears to be the most widely accepted mode of action (Oliver, 2004; Oliver *et al.*, 2004; Lin *et al.*, 2005).

In view of the above, the present study is original because it demonstrated that the therapy with MSCs could be an alternative for patients under chemotherapy treatment who developed ARF. Also, considering the fact that cancer is a growing public health concern and the majority of treatments involve aggressive chemical components, this kind of therapy could be an important alternative to improve the health quality of patients. Thus, considering that current therapies for ARF are merely palliative and that MSC therapy promotes the recovery of renal function in this model, we suggest that innovative/alternative therapies involving MSCs should be considered for clinical studies in humans to treat ARF.

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References

- Angeli JP, Ribeiro LR, Gonzaga ML, Soares S de A, Ricardo MP, Tsuboy MS, Stidl R, Knasmueller S, Linhares RS and Mantovani MS (2006) Protective effects of beta-glucan extracted from *Agaricus blazei* against chemically induced DNA damage in human lymphocytes. *Cell Biol Toxicol* 22:285-291.
- Angeli JP, Ribeiro LR, Angeli JL and Mantovani MS (2009a) Protective effects of beta-glucan from barley against benzo[a]pyrene-induced DNA damage in hepatic cell HepG2. *Exp Toxicol Pathol* 61:83-89.
- Angeli JP, Ribeiro LR, Bellini MF and Mantovani MS (2009b) Beta-glucan extracted from the medicinal mushroom *Agaricus blazei* prevents the genotoxic effects of benzo[a]pyrene in the human hepatoma cell line HepG2. *Arch Toxicol* 83:81-86.
- Best TM, Fiebig R, Corr DT, Brickson S and Ji L (1999) Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. *J Appl Physiol* 87:74-82.
- Brickson S, Hollander J, Corr DT, Ji LL and Best TM (2001) Oxidant production and immune response after stretch injury in skeletal muscle. *Med Sci Sports Exerc* 33:2010-2015.
- Brodie JC and Humes HD (2005) Stem cell approaches for the treatment of renal failure. *Pharmacol Rev* 57:299-313.
- Butterfield TA, Best TM and Merrick MA (2006) The dual roles of neutrophils and macrophages in inflammation: A critical balance between tissue damage and repair. *J Athl Train* 41:457-465.
- Camargo JLV, Oliveira MLCS, Rocha NS and Ito N (1994) A detecção de substâncias cancerígenas em estudos experimentais. *Rev Bras Cancerol* 40:21-30.

- Choi SJ, Kim JK and Hwang SD (2010) Mesenchymal stem cell therapy for chronic renal failure. *Expert Opin Biol Ther* 10:1217-1226.
- Chorvatovicová D (1991) Suppressing effects of glucan on micro-muceli induced by Co60 in mice. *Strahlenther Onkol* 167:612-614.
- Chorvatovicová D, Machová E and Sandula J (1996) Effect of ultrasonicated carboxymethylglucan on cyclophosphamide induced mutagenicity. *Mutat Res* 371:115-120.
- Chorvatovicová D, Machová E and Sandula J (1998) Ultrasonication: The way to achieve antimutagenic effect of carboxymethyl-chitin-glucan by oral administration. *Mutat Res* 412:83-89.
- Cisneros RL, Gibson FC and Tzianabos AO (1996) Passive transfer of poly-(1-6)- β -glucotriosyl-(1-3)- β -glucopyranose glucana protection against lethal infection in an animal model of intra-abdominal sepsis. *Infect Immun* 64:2201-2205.
- Cline SD and Hanawalt PC (2003) Who's on first in the cellular response to DNA damage? *Nat Rev Mol Cell Biol* 4:361-372.
- De Flora S (1998) Mechanisms of inhibitors of mutagenesis and carcinogenesis. *Mutat Res* 402:151-158.
- Demir G, Klein HO, Mandel-Molinas N and Tuzuner N (2007) β -glucan induces proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer. *Int Immunopharmacol* 7:113-116.
- Di Luzio NR, Williams DL, Mcnamee RB, Edwards BF and Kitahama A (1979) Comparative tumor-inhibitory and antibacterial activity of soluble and particulate glucan. *Int J Cancer* 24:773-779.
- Dragsted LO, Strube M and Larsen JC (1993) Cancer-protective factors in fruits and vegetables: Biochemical and biological background. *Pharmacol Toxicol* 72:116-135.
- Dusse LMS, Vieira LM and Carvalho MG (2003) Nitric oxide revision. *J Bras Patol Med Lab* 39:343-350.
- Epicum P, Detry O, Weekers L, Bonvoisin C, Lechanteur C, Briquet A, Beguin Y, Krzesinski JM and Jouret F (2014) Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion. *Nephrol Dial Transplant* 29:1487-1493.
- Fearon ER and Jones PA (1992) Progressing toward a molecular description of colorectal cancer development. *FASEB J* 6:2783-2790.
- Fry AC and Farrington K (2006) Management of acute renal failure. *Postgrad Med J* 82:106-116.
- Gordon JA and Gattone 2nd VH (1986) Mitochondrial alterations in cisplatin-induced acute renal failure. *Am J Physiol* 250:F991-F998.
- Grimm PC, Nickerson P, Jeffery J, Savani RC, Gough J, McKenna RM, Stern E and Rush DN (2001) Neointimal and tubulointerstitial infiltration by recipient mesenchymal cells in chronic renal-allograft rejection. *N Engl J Med* 345:93-97.
- Hayashi M, Morita T, Kodama Y, Sofuni T and Ishidate Jr M (1990) The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat Res* 245:245-249.
- Hermeto LC, Oliveira RJ, Matuo R, Jardim PH, DeRossi R, Antonioli AC, Deffune E, Evaristo TC and Santana AE (2015) Evaluation of pH effects on genomic integrity in adipose-derived mesenchymal stem cells using the comet assay. *Genet Mol Res* 14:339-348.
- Hoste EA and Schurgers M (2008) Epidemiology of acute kidney injury: How big is the problem? *Crit Care Med* 36:S146-S151.
- Humphreys BD, Soiffer RJ and Magee CC (2005) Renal failure associated with cancer and its treatment: An update. *J Am Soc Nephrol* 16:151-161.
- Kada T, Inoue T and Namiki N (1982) Environmental desmutagens and antidesmutagens. In: Klekowski EJ (ed) *Environmental Mutagenesis and Plant Biology*. Praeger, New York, pp 137-151.
- Kada T and Shimoi K (1987) Desmutagens and bio-antimutagens: Their modes of action. *BioEssays* 7:113-115.
- Kaneno Y, Chihara G and Taguchi T (1989) Activity of lentinan against cancer and AIDS. *Int J Immunother* 4:203-213.
- Kobayashi H, Sugiyama C, Morikawa Y, Hayashi M and Sofuni T (1995) A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis assay. *MMS Communications* 2:103-115.
- Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, *et al.* (2006) Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 24:386-398.
- Kumar V, Sinha AK, Makkar HP, de Boeck G and Becker K (2012) Dietary roles of non-starch polysachharides in human nutrition: A review. *Crit Rev Food Sci Nutr* 52:899-935.
- Lange C, Tögel F, Itrich H, Clayton F, Nolte-Ernsting C, Zander AR and Westenfelder C (2005) Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int* 68:1613-1617.
- Lazarová M, Lábaj J, Kogan G and Slamenová D (2006) Carboxymethyl chitin-glucan enriched diet exhibits protective effects against oxidative DNA damage induced in freshly isolated rat cells. *Neoplasma* 53:434-439.
- Lin H, She Y, Cassileth B, Sirotnak F and Rundles SC (2004) Maitake beta-glucan MD-fraction enhances bone marrow colony formation and reduces doxorubicin toxicity in vitro. *Int. Immunopharmacology* 4:91-99.
- Luiking YC, Poeze M, Ramsay G and Deutz NE (2005) The role of arginine in infection and sepsis. *J Parenter Enteral Nutr* 29:70-74.
- Magnani M, Castro-Gomez RJH, Mori MP, Kyasne H, Gregório EP, Libos-Jr F and Cólus IMS (2011) Protective effect of carboxymethyl-glucan (CM-G) against DNA damage in patients with advanced prostate cancer. *Genet Mol Biol* 34:131-135.
- Manoharan K and Banerjee MR (1985) beta-Carotene reduces sister chromatid exchanges induced by chemical carcinogens in mouse mammary cells in organ culture. *Cell Biol Int Rep* 9:783-789.
- Mantovani MS, Bellini MF, Angelia JPF, Oliveira RJ, Silva AF and Ribeiro LR (2008) β -Glucans in promoting health: Prevention against mutation and cancer. *Mutat Res* 658:154-161.
- Masihi KN (2000) Immunomodulators in infectious diseases panoply of possibilities. *Int J Immunopharmacol* 22:1083-1091.
- McCord JM (1993) Human disease, free radicals, and the oxidant/antioxidant balance. *Clin Biochem* 26:351-357.

- Oliveira RJ, Ribeiro LR, Silva AF, Matuo R and Mantovani MS (2006) Evaluation of antimutagenic activity and mechanisms of action of beta-glucan from barley, in CHO-k1 and HTC cell lines using the micronucleus test. *Tox in Vitro* 20:1225-1233.
- Oliveira RJ, Matuo R, Silva AF, Matiazi HJ, Mantovani M and Ribeiro LR (2007) Protective effect of beta-glucan extracted from *Saccharomyces cerevisiae*, against DNA damage and cytotoxicity in wild-type (k1) and repair-deficient (xrs5) CHO cells. *Tox in Vitro* 21:41-52.
- Oliveira RJ, Salles MJ, da Silva AF, Kanno TYN, Lourenço ACS, Freiria GA, Matiazi HJ, Ribeiro LR and Mantovani MS (2009a) Effects of the polysaccharide β -glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. *Regul Toxicol Pharmacol* 53:164-173.
- Oliveira RJ, Baise E, Mauro MO, Pesarini JR, Matuo R, Silva AF, Ribeiro LR and Mantovani MS (2009b) Evaluation of chemopreventive activity of glutamine by the comet and the micronucleus assay in mice's peripheral blood. *Environ Toxicol Pharmacol* 28:120-124.
- Papazova DA, Oosterhuis NR, Gremmels H, van Koppen A, Joles JA and Verhaar MC (2015) Cell-based therapies for experimental chronic kidney disease: A systematic review and meta-analysis. *Dis Model Mech* 8:281-293.
- Patchen ML, D'Alesandro MM, Brook I, Blakely WF and Mac Vittie TJ (1987) Glucan: Mechanisms involved in its radioprotective effect. *J Leukocyte Biol* 42:95-105.
- Pinkerton pH and Dubé ID (1991) Chronic myeloid leukemia as a paradigm for oncogenesis. *Diagn Oncol* 1:288-297.
- Pitot HC (1993) The molecular biology of carcinogenesis. *Cancer* 72:962-970.
- Pitot HC and Dragan Y (1991) Facts and theories concerning the mechanisms of carcinogenesis. *FASEB J* 5:2280-2286.
- Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, *et al.* (1999) The Banff 97 working classification of renal allograft pathology. *Kidney Int* 55:713-723.
- Ren L, Zhou Q, Hou M, Qiu M, He J and Chen H (2002) Monitoring of the renal function changes during chemotherapy based on high-dose cisplatin in patients with lung cancer. *Zhongguo Fei Ai Za Zhi* 5:363-365.
- Salvadori DMF, Ribeiro LR and Fenech M (2003) Teste do micronúcleo em células humanas *in vitro*. In: Ribeiro LR, Salvadori DMF and Marques EK (eds) *Mutagênese Ambiental*. ULBRA, Canoas, pp 201-219.
- Serpeloni JM, Batista BL, Angeli JP, Barcelos GR, Bianchi M de L, Barbosa Jr F and Antunes LM (2013) Antigenotoxic properties of chlorophyll b against cisplatin-induced DNA damage and its relationship with distribution of platinum and magnesium *in vivo*. *J Toxicol Environ Health A* 76:345-353.
- Silva AF, Oliveira RJ, Niwa AM, D'Epiro GFR, Ribeiro LR and Mantovani MS (2012) Anticlastogenic effect of β -glucan, extracted from *Saccharomyces cerevisiae*, on cultured cells exposed to ultraviolet radiation. *Cytotechnology* 65:41-48.
- Singh NP, McCoy MT, Tice RR and Schneider EL (1988) A simple technique for quantification of low levels of DNA damage in individual cells. *Exp Cell Res* 175:184-191.
- Slamenová D, Lábaj J, Krizková L, Kogan G, Sandula J, Bresgen N and Eckl P (2003) Protective effects of fungal (1 \rightarrow 3)- β -D-glucan derivatives against oxidative DNA lesions in V79 hamster lung cells. *Cancer Lett* 198:153-160.
- Sugimura T (1992) Multistep carcinogenesis: A 1992 perspective. *Science* 258:603-607.
- Tögel F, Isaac J, Hu Z, Weiss K and Westenfelder C (2005) Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289:F31-F42.
- Tohamy AA, El-Ghor AA, El-Nahas SM and Noshay MM (2003) β -Glucan inhibits the genotoxicity of cyclophosphamide, adriamycin and cisplatin. *Mutat Res* 541:45-53.
- Torrinhas RSM, Campos LDN and Mazza RPJ (2006) Metodologia na pesquisa de dieta, nutrição e câncer: Estudos experimentais em modelos animais e culturas de células. In: Waitzberg DL (ed) *Dieta, Nutrição e Câncer*. Ateneu, São Paulo, pp 665-674.
- Turnbull JL, Patchen ML and Scadden DT (1999) The polysaccharide, PGG-glucan, enhances human myelopoiesis by direct action independent of and additive to early-acting cytokines. *Acta Haematol* 102:66-71.
- Vieira MH, Oliveira RJ, Eça LP, Pereira IS, Hermeto LC, Matuo R, Fernandes WS, Silva RA and Antoniolli AC (2014) Therapeutic potential of mesenchymal stem cells to treat Achilles tendon injuries. *Genet Mol Res* 13:10434-10449.
- Xiao Z, Trincado CA and Murtaugh MP (2004) β -Glucan enhancement of T cell IFN-gamma response in swine. *Vet Immunol Immunopathol* 102:315-320.
- Waters MD, Brady AL, Stack HF and Brockman HE (1990) Antimutagenicity profiles for some model compounds. *Mutat Res* 238:57-85.
- Wen X, Murugan R, Peng Z and Kellum JA (2010) Pathophysiology of acute kidney injury: A new perspective. *Contrib Nephrol* 165:39-45.
- Yokoo T, Sakurai K, Ohashi T and Kawamura T (2003) Stem cell gene therapy for chronic renal failure. *Curr Gene Ther* 3:387-394.
- Zerbini G, Piemonti L, Maestroni A, Dell'Antonio G and Bianchi G (2006) Stem cells and the kidney: A new therapeutic tool? *J Am Soc Nephrol* 17:S123-S126.
- Zimmerman JW, Lindermuth J, Fish PA, Palace GP, Stevenson TT and DeMong DE (1998) A novel carbohydrate-glycosphingolipid interaction between a β -(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. *J Biol Chem* 273:22014-22020.

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