The Enhancement Effect of Mesenchymal Stem Cells on Nephrotoxicity Induced by Cisplatin in Albino Rats


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Abstract

Background: Cisplatin is one of the most effective and potent anticancer drugs. It is used in the treatment of a wide variety of both pediatric and adult malignancies. However, the chemotherapeutic use of cisplatin is limited by its serious side effects such as nephrotoxicity. Bone marrow derived mesenchymal stem cells (MSCs) have shown great potential in cell therapy of solid organs.

Methods: This work included thirty rats which were divided equally into three groups: Healthy control group, nephrotoxicity group induced by cisplatin, nephrotoxicity group received MSCs. Serum levels of tumor necrosis factor alpha (TNF α) by ELISA and serum levels of urea and creatinine by colorimetry were performed in all groups.

Results: Serum levels of TNF α, urea and creatinine were decreased in the treated group compared to the diseased group.

Conclusion: Administration of MSCs improves nephrotoxicity as evidenced by the biochemical marker of inflammation (TNF α) and kidney function tests.

Key Words: Nephrotoxicity – Inflammation – Cisplatin – MSCs.

Introduction

KIDNEY diseases are considered a public health issue worldwide. They can become chronic, lead to increased risk of cardiovascular disease and raise healthcare costs. In addition, they contribute to approximately 850,000 deaths each year, making them the 12th leading cause of death. The ageing population and advanced life expectancy have contributed to the increased prevalence of chronic diseases, including chronic kidney disease. However, people can develop acute kidney injury (AKI), which is associated with diverse causes, such as burns, shock, drug toxicity, sepsis, trauma and severe diarrhea, at any age [1].

Exposure to drugs often results in toxicity in kidney which represents the major control system maintaining homeostasis of body and thus is especially susceptible to xenobiotics. Understanding the toxic mechanisms for nephrotoxicity provides useful information on the development of drugs with therapeutic benefits with reduced side effects. Biomarkers have been identified for the assessment of nephrotoxicity. The discovery and development of novel biomarkers that can diagnose kidney damage earlier and more accurately are needed for effective prevention of drug-induced nephrotoxicity [2].

Cis-Diamminedichloroplatinum (cisplatin) is a highly effective antineoplastic agent, and the cisplatin-based combination chemotherapy regimens are currently used as the front-line therapy in the treatment of different types of cancer [3]. However, cisplatin use is limited because of its side effects in normal tissues, mainly the kidney leading to nephrotoxicity [4,5].

Stem cells are a unique type of cell that forms the basis of the development, growth and survival of a living organism [6]. Stem cells hold the remarkable capacity of self-renewal and differentiation into more specialized cell lineages, and thus constitute a promising resource in regenerative medicine for the generation of appropriate cell types in cell replacement therapy. Stem cell research, accordingly, has become a highly vigorous and rapidly evolving field in life sciences [7].

In nephrology, stem cell therapy holds a great promise for the repair of injured tissues and organs, including the kidney [8]. Bone marrow-derived mesenchymal stem cells (MSCs) may have the capacity to migrate to the injured kidney and contribute to tubule epithelium regeneration and renal
function repair without fusing with resident tubular cells [9].

The aim of this work was to evaluate the effect of MSCs on cisplatin induced nephrotoxicity in albino rats.

**Material and Methods**

This study included thirty male rats inbred strain (Cux 1: HEL 1) of matched age and weight (6 months-1 year & 120-150gm). Animals were inbred in the experimental animal unit, Faculty of Medicine, Cairo University from July 2014 – July 2015. Rats were maintained according to the standard guidelines of Institutional Animal Care and Use Committee and after Institutional Review Board approval. Animals were fed a semi-purified diet that contained (gm/kg): 200 casein, 555 sucrose, 100 cellulose, 100 fat blends, 35 vitamin mix, and 35 mineral mix.

This study involved the following:

**I- Isolation, propagation, identification and labeling of bone marrow-derived MSCs from rats:**

**A- Isolation and propagation of BM-derived MSCs from rats:**

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male rats with Dulbecco’s modified Eagle’s medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin/streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12-14 days as primary culture. Media was changed every 2-3 days.

When large colonies developed (80-90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50cm2 culture flasks (Falcon). The resulting cultures were referred to as first passage cultures [10]. On day 14, the adherent colonies of cells were trypsinized, and counted.

**B- Identification of BM-derived MSCs from rat:**

Cells were identified as being MSCs by their morphology, adherence, by detection of CD 90, CD 105, CD 34 which is one of the surface markers of rat mesenchymal stem cells in MSC culture and their power to differentiate into osteocytes [11] and chondrocytes [12].

Differentiation into osteocytes was achieved by adding 1-1000nM dexamethasone, 0.25mM ascorbic acid, and 1-10mM beta-glycerophosphate to the medium. Differentiation of MSCs into osteoblasts was achieved through morphological changes, Alizarin red staining of differentiated osteoblasts. Differentiation into chondrocyte was achieved by adding 500ng/ml bone morphogenic protein-2 (BMP-2; R&D Systems, USA) and 10ng/ml transforming growth factor b3 (TGFb3) (Peprotech, London) for 3 weeks [12]. In vitro differentiation into chondrocytes was confirmed by morphological changes, Alcian blue staining of differentiated chondrocytes. Flow cytometric analysis of cultured MSCs surface markers was done.

**C- Labeling of stem cells with PKH26:**

PKH26 is a red fluorochrome. It has excitation (551nm) and emission (567nm) characteristics compatible with rhodamine or phycoerythrin detection systems. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity, and are ideal for in vitro cell labeling, in vitro proliferation studies and long term, in vivo cell tracking.

In the current work, undifferentiated MSCs were labeled with PKH26 according to the manufacturer’s recommendations (Sigma, Saint Louis, Missouri, USA). Cells were injected intravenously into rat tail vein. After one month, kidney tissue was examined with a fluorescence microscope to detect the cells stained with PKH26.

**II- Preparation of experimental animal:**

**Animals were divided equally into 3 groups:**

- **Group 1:** Ten healthy control rats (negative control group).
- **Group 2:** Ten rats received a single dose of cisplatin (7mg/kg b.w.,i.p) to induce acute nephrotoxicity [13].

Nephrotoxicity was determined by serum urea and creatinine levels and by histopathological examination.

- **Group 3:** Ten rats received MSCs (5x105 cells as a single dose in tail vein) after nephrotoxicity induction with cisplatin [14].

Venous blood was collected from the retroorbital vein from rats of all groups. At the planned time animals were sacrificed.
Kidney tissue was harvested for assessment of TNF alpha by Elisa Supplied by Biospes, China. Assessment of kidney functions by measurement of urea and creatinine in serum by colorimetry using QuantiChromTM Urea Assay kit (DIUR-500) [15] and QuantiChromTM creatinine Assay Kit [16] respectively.

**Statistical analysis:**

Data were coded and entered using the statistical package SPSS version 22. Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using unpaired \( t \)-test when comparing 2 groups and analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than 2 groups. \( p \)-values less than 0.05 were considered as statistically significant.

**Results**

As shown in Fig. (1) the serum level of urea was significantly increased in the cisplatin (diseased) group compared to the healthy control group (\( p < 0.001 \)), whereas the treated group that received MSCs (\( p < 0.05 \)) showed significant decrease compared to the diseased group. As shown in Fig. (2) the serum level of creatinine was significantly increased in the diseased group compared to the control group (\( p < 0.001 \)), whereas the treated group showed significant decrease compared to the diseased group (\( p < 0.001 \)).

As seen in Fig. (3) mean levels of TNF alpha showed significant increase in the diseased group compared to the control group (\( p < 0.001 \)) but significant decrease in the treated group compared to the diseased group (\( p < 0.001 \)).

**Discussion**

General mechanisms that cause nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy [17]. Cisplatin is one of the most commonly used present day chemotherapeutic agents. It is used to treat a wide range of cancers including head and neck, lung, gastrointestinal tract, ovarian and genitourinary cancers. However, dose-limiting toxicity is often associated with cisplatin. It is known that cisplatin works more effectively with dose escalation, but significant risk for nephrotoxicity is often associated with higher doses [18].
New hopes for curing acutely damaged organs come from stem cell-based treatment exploiting stem cell’s peculiar properties of tropism and regenerative capability. In this context,MSCs represent an attractive tool because of their beneficial effects on tissue repair in experimental models of myocardial infarction, neurologic disease, and more recently, in experimental acute kidney injury [19].

Our results are similar to those of [20] who demonstrated that cisplatin treated group showed high significant elevation of serum levels of urea and creatinine as compared to the control group. On the other hand, MSCs/cisplatin treated animals revealed high significant reduction of the elevated levels of serum urea and creatinine as compared to the diseased group. However serum urea and creatinine were not completely ameliorated and were still significantly elevated as compared to control [21] found that in the cisplatin induced acute renal failure group, urea, creatinine and uric acid levels were increased and the percentage of such While, after stem cell transplantation they decreased significantly.

Our results are in accordance with the work of [3] who showed that TNFα gene expression and circulating levels were increased in the cisplatin induced nephrotoxicity animal model compared to the control group. These results are in accordance with [22] who confirmed that the anti-inflammatory action of MSCs is exerted by suppressing the release of TNF-α from renal tubular cells; the inflammatory cytokine mediating cisplatin-induced renal toxicity.

Conclusion:

The treatment with MSCs has been shown to improve nephrotoxicity as evidenced by biochemical markers.

References


