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Therapeutic efficacy of bone marrow derived mesenchymal stromal cells versus losartan on adriamycin-induced renal cortical injury in adult albino rats

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Abstract

Background. Renal disease is a major health problem. Recent studies have reported the efficacy of stem cell therapy in nephropathy animal models. *Aim of the work.* This study was designed to investigate the therapeutic effectiveness of bone marrow–derived mesenchymal stromal cells (MSCs) versus losartan in the treatment of renal alterations induced by adriamycin (ADR). *Materials and methods.* Thirty-five adult male albino rats were divided into four groups. Group I was the control group. Group II (adriamycin-treated group),which included ten rats that were injected with a single dose of adriamycin (15 mg/kg) intraperitoneally, was subdivided into subgroup IIa and IIb and they were sacrificed 1 week and 5 weeks after adriamycin injection, respectively. Group III was the adriamycin + losartan–treated group and 1 week after adriamycin injection five rats received 10 mg/kg of losartan orally and daily for 4 weeks. Group IV was the adriamycin + MSC–treated group); five rats were injected with adriamycin as group II then supplied with MSCs at a dose of 1×10^6 cells suspended in 0.5 mL of phosphate-buffered saline (PBS) per rat in the tail vein 1 week after adriamycin injection. Rats of this group were sacrificed 4 weeks after the stem cell injection. Blood urea nitrogen and serum creatinine were measured. Samples from renal cortex were processed for light and electron microscope examination. As regards light microscope, sections were stained with hematoxylin and eosin (H-E), periodic acid–Schiff (PAS), masson trichrome, proliferating cell nuclear antigen (PCNA) and Caspase-3 immunohistochemical stains. Morphometrical and statistical analyses were also conducted. *Results.* Examination of adriamycin-treated group revealed deterioration of renal functions and various degrees of renal structural alterations as vacuolated cytoplasm, dark nuclei and detached epithelial lining. Administration of losartan partially improved ADR-induced kidney dysfunction, whereas MSCs denoted a more ameliorative role evidenced by structural and functional recovery. *Conclusion.* MSCs have a relevant therapeutic potential against ADR-induced renal damage. MSCs may accomplish this role by decreasing caspase-3 expression and increasing proliferating cell nuclear antigen staining which influence the regeneration of the kidney.

Key Words: *adriamycin, caspase-3, proliferating cell nuclear antigen, rats, renal cortex, stem cells*

Introduction

Kidney diseases are emerging as a global threat to public health that requires renal replacement therapy such as dialysis or renal transplantation. Owing to increasing incidence and the high costs of treatment of kidney diseases, progression of new approaches such as cell therapy has been developed [1]. Adriamycin is one of the anti-tumor drugs with a very wide spectrum of activity in human cancers but its clinical use has serious drawbacks. Adriamycin is a well-known inducer of renal injury in rodents, which mirrors that seen in human chronic kidney disease (CKD) [2].

Losartan belongs to a class of blood pressure medications called angiotensin II receptor blockers (ARBs). ARBs have been used as first-line therapies to reduce the progression of CKD [3]. Considerable gains have been obtained in retarding progression of CKD by renin-angiotensin system blockade in a significant proportion of patients; whereas, the therapeutic goal of arresting CKD progression to end-stage renal disease remains unfulfilled [4].

Mesenchymal stromal cells (MSCs) have been the focus of great interest in regenerative medicine for their ability to migrate to the site of injury, as well as for their multilineage differentiation potential and their straightforward *in vitro* expansion. MSCs are currently used in clinical trials for treating a wide range of tissue injury of different organs (heart, kidney, lung and liver) [5].

Proliferating cell nuclear antigen (PCNA) is a stable nuclear protein and represents a reliable

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determination of proliferative activity [6]. It is expressed in late G1 and expressed maximally during S-phase of the cell cycle and its rate of synthesis with time correlated with the proliferative rate of cells [7]. PCNA also serves as a key factor in many essential cellular processes, such as DNA replication, DNA repair, DNA damage and cell survival [8].

Caspases are a group of enzymes that are involved in the regulation of apoptosis resulting in the classical apoptotic features. Caspase-3 activation occurs in response to a variety of apoptotic inducers that activate endonuclease and induce DNA fragmentation [9].

Studies remain scarce about the effect of transplanted stem cells on renal structure and function in experimentally induced nephropathy. Therefore, this study was designed to investigate the therapeutic potential of MSCs on adriamycin-induced renal injury in rats using biochemical and histological approaches and to compare the effectiveness of stem cell therapy versus losartan in correcting the histological alterations detected in the renal cortex of adult male albino rats after adriamycin injection.

Materials and methods

Animals

Thirty-five adult healthy male albino rats (3–5 months) weighing 180–200 g were used in this study. They were obtained from the breeding animal house, Faculty of Medicine, Zagazig University. The experimental protocol was approved by the Zagazig University Research Ethics Committee.

The animals were kept in the animal house for 1 week in stainless steel cages to be acclimatized to the new environment before the experiment. The animals were maintained in accordance with the guidelines of the stem cell research unit in the central laboratory, Zagazig University. Throughout the duration of the experiment, the rats were housed in room temperature with normal light/dark cycles. They were allowed *ad libitum* access to food and water.

Chemicals

Adriamycin (Adricin) was obtained in the form of vials; each vial contained 50 mg of Adriamycin, manufactured by EIMC United Pharmaceuticals (EUP).

Losartan (Losartan potassium) was obtained in the form of film-coated tablets; each tablet contained 100 mg of losartan manufactured by Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO).

Bone marrow–derived-MSCs (BM-MSCs) labelled with PKH-26 (red fluorescence cell linker) were provided from the Biochemistry Department, Kasr Al-Ainy Medical School.

BM-MSCs were harvested by flushing the tibiae and femurs of 6-week-old male albino rats with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. Nucleated cells were isolated with a density gradient and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin. Cells were incubated at 37°C in 5% humidified CO_2 for 12–14 days, until formation of large colonies (80–90% confluence). The culture was washed with phosphate-buffered saline (PBS) and released with 0.25% trypsin in 1 mL ethylenediaminetetraacetic acid (EDTA; 5 min at 37°C). After centrifugation, the cells were resuspended with serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon) [10]. MSCs in culture were characterized by their adhesiveness and fusiform shape [11].

Regarding labeling of stem cells with Paul Karl Horan 26 (PKH-26; red fluorescence cell linker), MSCs were harvested during the second passage and were labeled with PKH-26 dye [12]. Cells were centrifuged and washed twice in serum-free medium. Cells were pelleted and suspended in dye solution.

Experimental protocol

The animals were classified into 4 main groups. Group I (control group) included 15 rats that were equally subdivided into three subgroups (five rats each). Subgroup Ia (negative control group): the animals of this group received no treatment. Subgroup Ib: the animals of this group received a daily dose of 1 mL of distilled water orally through stomach tube for 4 weeks as a vehicle of losartan [13]. Subgroup Ic: the animals of this group were injected with a single dose of 0.5 mL of PBS in the tail vein as a vehicle of stem cells [14]. The animals of each subgroup were sacrificed with their corresponding experimental group.

Group II (adriamycin-treated group) included ten rats that were injected with a single dose of adriamycin (15 mg/kg) intraperitoneally [15]. The animals of this group were subdivided into two subgroups. Subgroup IIa included two rats that were sacrificed 1 week after adriamycin injection to ensure renal cortical injury. Subgroup IIb included eight rats that were sacrificed 5 weeks after adriamycin injection to ensure persistence of renal cortical injury and exclude spontaneous recovery.

Group III (adriamycin group treated with losartan) included five rats. One week after adriamycin injection, rats received 10 mg/kg of losartan dissolved in 1 mL of distilled water orally through a stomach tube daily for 4 weeks then sacrificed using ether inhalation [16].

Group IV (adriamycin group treated with MSCs) included five rats that were injected with adriamycin