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Therapeutic efficacy of human umbilical cord mesenchymal stem cells transplantation against renal ischemia/reperfusion injury in rats

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ABSTRACT

Background: Acute kidney injury (AKI) is a common clinical problem raising the urgent needs to develop new strategies for treatment. The present study investigated the therapeutic potential of human umbilical cord – mesenchymal stem cells (HUC-MSCs) transplantation against renal ischemia/reperfusion injury (IRI) in rats.
Methods: Twenty four male Wistar rats were assigned into two main groups, sham group (control group) and I/R group. I/R group was injected in the tail vein with either phosphate buffer saline (PBS) or HUC-MSCs.
Results: The HUC-MSCs improved kidney injury induced by I/R as demonstrated by enhancement of the kidney function via decreasing serum levels of creatinine, urea and uric acid. The therapeutic efficacy of HUC-MSCs were found to be mediated through anti-oxidant activity as indicated by significant reduction in total malondialdehyde (MDA) and significant increment in the levels of reduced glutathione (GSH), catalase (CAT) and glutathione-S-transferase (GST).
Conclusion: The present work suggests that HUC-MSCs may be an effective therapeutic agent against renal IRI. The recorded data showed improvement of renal functions and urine albumin in HUC-MSCs than IRI group with positive antioxidant efficacy of HUC-MSCs through scavenging free radicals and supporting the antioxidant enzymes.

1. Introduction

Acute kidney injury (AKI) is a common clinical problem with significant morbidity and mortality. Renal ischemia/reperfusion injury (IRI) is the most common cause of AKI (Afyouni et al., 2016; Migita et al., 2016). Indeed, AKI is characterized by rapid and potentially reversible decline in renal function (Freitas et al., 2015). Renal IRI is a complicated process in which the kidney is subjected to morphological and functional damage during the ischemic phase and undergoes further insult during reperfusion (Elshiekh et al., 2015). The severity of the injury depends on the duration and the extent of ischemia and subsequent reperfusion. Reactive oxygen species (ROS) are important mediators exerting toxic effects on various organs, including kidney during ischemia-reperfusion injury (Karahan et al., 2016). Interruption of blood flow to the kidney and the subsequent reperfusion lead to an acute oxidative stress response that may cause the generation of ROS. It has been reported that, ROS cause persistent vasoconstriction and hence a decreased glomerular filtration rate (Sutton et al., 2002). Moreover, ROS are able to react with proteins, lipids, nucleic acids, and carbohydrates contributing to cell apoptosis and necrosis (Bonventre and Weinberg, 2003). Thus inactivating ROS might be an effective strategy to counteract renal IRI. The key to recovery of renal function following AKI is the repair of the tubular basement membrane by proliferating and differentiating renal tubular epithelial cell. An increasing number of studies suggest that stem cell therapy could have a positive impact on AKI (Večerić-Haler et al., 2016; Liu et al., 2016).

Mesenchymal stem cells (MSCs) are clinically useful due to their capacity for self-renewal, their immunomodulatory properties and tissue regenerative potential (Heo et al., 2016). Moreover, MSCs are recognized as a promising tool to improve renal recovery in acute kidney injury (Si et al., 2015). Umbilical cord blood (UCB) reportedly contains stem cells, which have been widely used as a hematopoietic source (Chung et al., 2016). The isolation of human umbilical cord MSCs (HUC-MSCs) from the whole umbilical cord

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provides an extensive and new source of MSCs; they exert beneficial effects against renal ischaemia-reperfusion injury in rats (Qiu et al., 2014). Ayatollahi et al. (2014) showed that bone marrow-derived MSCs promote an antioxidant response and support the potential of using MSCs transplantation as an effective treatment modality for liver disease. Moreover, Kim et al. (2015) reported that the antioxidant and anti- inflammatory effects of intravenously injected adipose derived MSCs (AD-MSCs) in dogs with acute spinal cord injury results suggest that early intravenous injection of AD-MSCs after acute spinal cord injury (SCI) may prevent further damage through enhancement of anti oxidative and anti- inflammatory mechanisms. Our study aimed to study the beneficial efficacy of HUC-MSCs in alleviating IRI of the kidney through employing a battery of laboratory markers and evaluating histological architecture of the kidney rat before and after this therapeutic modality.

2. Materials and methods

2.1. Collection of Wharton jelly human umbilical cord blood (HUCB) samples

HUCB samples were obtained immediately after delivery (as with elective caesarian section only), the umbilical cord of human candidate is clamped, sterilized by betadine (povidone lodine) and then cut by a sterile scissors. The cord is placed in a sterile cup filled with 50 ml sterile 0.9% normal saline and 0.5 ml antifungal (Amphotericin B) and 0.5 ml antibacterial drug (penicillin-streptomycin)(pen– strep) at 4° C until processing.

2.2. Sample transportation

The cup is transported inside a closed container containing an ice bag in the floor of the container and a thermometer is placed to assure the temperature did not exceeding 8 °C. The container is transported to the lab to be processed. Typically, the cord is processed within 12–24 h of birth.

2.3. Sample processing in the lab

The cord is handled in an aseptic fashion and processed in a Type II Biological Safety Cabinet. Before starting to process the cord the laminar air flow cabinet should be cleaned and sterilized by alcohol. Under the laminar air flow we prepared 2 cups one containing 70% alcohol (ethyl alcohol) and the other one containing 0.9% normal sterile saline. The cord was immersed in the 70% alcohol cup with forceps for one minute and in the sterile saline (0.9% normal saline) cup for one minute.

The umbilical cord is placed in the sterile petri dish then the cord is dissected and minced into several pieces called (explants) by sterile blade. Explants vary in size from 2 to 3 mm up to 4–5 mm. Explants are cultured in complete media for a total of 3–4 weeks in 4 T-75 sterile flasks. All steps are hold under the laminar air flow. Complete media is made of: Alpha Modified Eagles medium (AMEM) 45 ml, fetal bovine serum (FBS)5 ml, penicillin – streptomycin 0.5 ml and fungazole (amphotrycin B) 0.5 ml. Flasks are incubated in a 5% CO2 incubator at 37 °C for 48 h. The media was changed after 48 h with 10 ml fresh culture media\added to each flask. Incubation for 4 weeks in 5% CO2 incubator with changing the media with 10 ml fresh culture media was done weekly for 4 weeks. After the cells reaching 80–90% confluence enzymatic separation of cells by pre warming 4 ml Trypsin (0.2%) in phosphate buffer saline (PBS) to 37 °C was done. A scrubber was used to dissociate cells. The scrubbed cells were added in 50 ml sterile tube. And then was centrifuged at 1200 rpm for 10 min. The cell number was counted (about 70 millions/ml). Finally the cells were suspended in PBS ready for injection.

2.4. Experimental animals

The experimental animals used in this study were the male albino Wistar rats (*Rattus norvegicus*) weighing (150–160 gm). The rats were obtained from the National Research Center (NRC, Dokki, Giza). They were grouped and housed in polyacrylic cages (five animals per cage) in the well–ventilated animal house of the Zoology Department, Faculty of Science, Cairo University. Rats were given food and water ad libitum. Rats were maintained in a friendly environment of a 12 h/12 h light-dark cycle at room temperature (22–25 °C).

2.5. Ethical consideration

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/F/PHY/04/13). All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

2.6. Induction of unilateral renal ischemia reperfusion (I/R)

Male Wistar rats were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). A midline abdominal incision was made to expose the left kidney. Blood supply to the kidney was interrupted by clamping the left renal artery using artery clamp for 1 h. Ischemia was confirmed by the blanching of the kidney. After 1 h the clamp was removed and reperfusion was confirmed visually. The wound was then closed in two layers with silk suture and the animals were allowed to recover with free access to food and water according to the method described by Savransky et al., 2006

2.7. Transplantation of HUC-MSCs cells

Two hundred μl of PBS solution was added to the HUC mononuclear cells pellet for injection in the rat tail vein at a dose of 1×106 cells/rat for (IRI + HUC-MSCs) group.

Table 1
Therapeutic efficacy of human umbilical cord mesenchymal stem cells (HUC-MSCs) transplantation on kidney functions and urine albumin following renal ischemia-reperfusion injury (IRI) in rats.

Group	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Urine albumin (mg/dl)
Sham	0.65 ± 0.02	77.66 ± 3.19	1.18 ± 0 0.10	41.53 ± 1.48
IRI	1.02 ± 0.05	222.288 ± 6.32	1.82 ± 0 0.06	156.71 ± 1.56
IRI + HUC – MSCs	0.60 ± 0.04	46.50 ± 1.43	1.25 ± 0 0.09	48.10 ± 2.02
% improvement	41.18%	79.08%	31.32%	69.31
P value	<0.001 HS	<0.001 HS	<0.001 HS	<0.001 HS

HS: Highly significant.