The Role of Mesenchymal Stem Cells versus Its Conditioned Medium on Cisplatin Induced Renal Injury in Adult Albino Rat

Thesis

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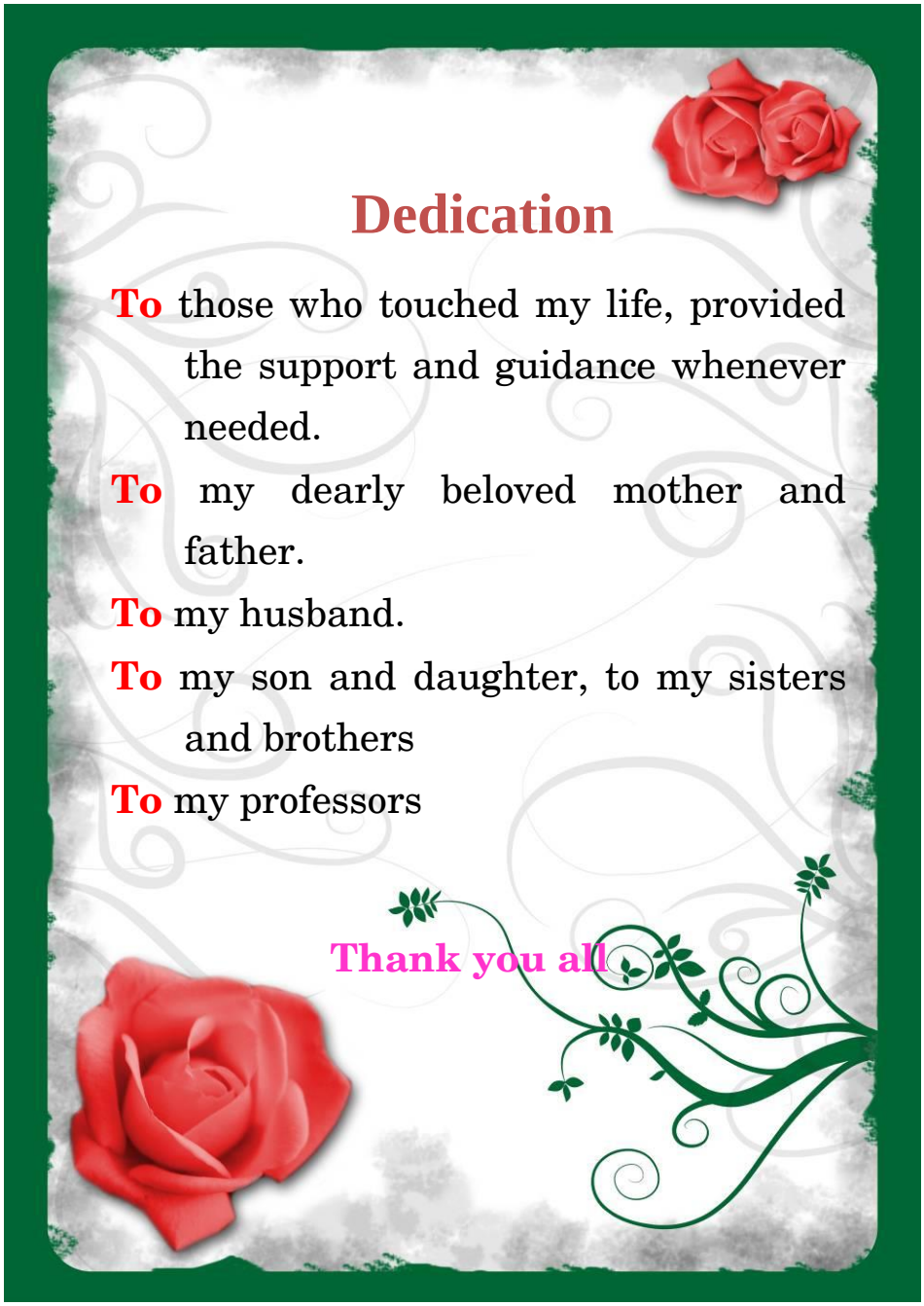

Dedication

To those who touched my life, provided the support and guidance whenever needed.

To my dearly beloved mother and father.

To my husband.

To my son and daughter, to my sisters and brothers

To my professors

Thank you all

List of Contents

List of Abbreviations

ABC	:	Avidin Biotin Peroxidase complex
Ad-MSCs	:	Adipose tissue-derived mesenchymal stem cells
AKI	:	Acute kidney injury
ATN	:	Acute tubular necrosis
BMSCs	:	Bone marrow-derived mesenchymal stem cells
bp	:	Base pairing
CD	:	Cluster of differentiation
CDDP	:	Cis-diamminedichloroplatinum
CM	:	Conditioned medium
Ctr1	;	Copper transporter 1
DAB	:	Diaminobenzidine
DAMPs	:	Damage associated molecular pattern
DCT	:	Distal convoluted tubule
DMEM	:	Dulbecco ′s Modified Eagles Medium
DNA	:	Deoxyribonucleic acid
ECM	:	Extracellular matrix
EDTA	:	Ethylene diammine tetracetic acid
EMT	:	Epithelial mesenchymal transition
ESCs	:	Embryonic stem cells
FBS	:	Fetal bovine serum
GFR	:	Glomerular filtration rate
GGT	:	Gamma glutamyle transpeptidase
gm	:	Gram
H_2O_2	:	Hydrogen peroxide

List of Abbreviations (Cont.)

HA	:	Hyaluronic acid
HGF	:	Hepatocyte growth factor
ICAM1	:	Intercellular adhesion molecule 1
IGF-1	:	Insulin like growth factor-1
IL	:	Interleukin
IR	:	Ischemia reperfusion
kg	:	Kilogram
m^2	:	Square-meter
mg	:	Milligram
MHC	:	Major histocompatibility complex
ml	:	Milliliter
MSCs	:	Mesenchymal stem cells
OCT2	:	Organic cation transporter 2
PAS	:	Periodic acid Schiff reagent
PBS	:	Phosphate buffered saline
PCNA	:	Proliferating cell nuclear antigen
PCR	:	Polymerase chain reaction
PCT	:	Proximal convoluted tubule
PLA	:	Processed lipoaspirate cell
PTECs	:	Proximal tubular epithelial cells
ROS	:	Reactive oxygen species
RPM	:	Round per minute
SPSS	:	Statistical Package for the Social Sciences
SRY	:	Sex determining region of y chromosome
TGF	:	Transforming growth factor
TLR4	:	Toll like receptor 4
TNF	:	Tumor necrosis factor
VCAM1	:	Vascular adhesion molecule 1

List of Abbreviations (Cont.)

VEGF	:	Vascular endothelial growth factor
VSELs	:	Very small embryonic like stem cells
ZO-1	:	Zonula occludens protein-1
α-SMA	:	Alpha-smooth muscle actin
µl	:	Microliter
µm	:	Micrometer
MET	:	Mesenchymal epithelial transition
ER	:	Endoplasmic reticulum
miR	:	Micro-RNA

Abstract

Background and aim of the study: Acute kidney injury (AKI) is a syndrome of rapidly declining renal function. This study was conducted to evaluate the capability of BMSCs versus its conditioned medium in minimizing the cisplatin induced renal injury.

Methods: Sixty adult female albino rats were divided into 4 groups. Group I (the control group), Group II (cisplatin treated group), Group III (stem cell treated group), Group IV (conditioned medium treated group). Rats of all groups were sacrificed on 4^{th} day of the experiment. Renal specimens were prepared for histological and immunohistochemical techniques. Morphometrical and statistical analysis were performed.

Results: Light microscopic examination revealed necrotic and apoptotic changes in renal tubules. Sloughing of epithelial lining of some tubules to the lumen of tubules with formation of acidophilic casts were detected. Fibrous tissue could be detected in the interstitium and lumen of some tubules. Injection of BMSCs resulted in improvement of renal structure. Injection of conditioned medium (CM) was less effective than BMSCs in treatment of AKI.

Conclusion: BMSCs injection is preferable than CM in the treatment of cisplatin induced AKI.

Keywords: Acute kidney injury, bone marrow-derived mesenchymal stem cells, conditioned medium.

Introduction

Acute kidney injury (AKI) is a syndrome of rapidly declining renal function induced by a number of different insults **(Mingeot-Leckercq and Tulkens, 1999)**. The mortality rate of hospital acquired AKI currently ranges from 30 to 80%. Recent dialysis techniques, continuous renal replacement therapy and others have no significant impact on overall mortality. Furthermore, the efforts to develop new pharmacological therapies have been largely unsuccessful **(Nash et al., 2002)**.

Cisplatin is an anti-neoplastic drug used in the treatment of many solid-organ cancers including those of head, neck, lung, testis, ovary and breast (**Hartmann and Lipp, 2003).** While cisplatin toxicities include ototoxicity, gastrotoxicity and allergic reactions, its main dose-limiting side effects is nephrotoxicity **(Sastry and Kelle, 2005).** Now, it is recognized that the prevalence of cisplatin nephrotoxicity is high occurring in about one-third of patients undergoing cisplatin treatment **(Zamble and Lippard, 1995).**

Cell-based therapeutic approaches have several potential advantages over specific drugs or growth factors in treatment of complex disorders such as acute kidney injury **(Herrera et al., 2004 and Bi et al., 2007).** The field of regenerative medicine seeks to repair, replace or regenerate tissues and organs damaged by injury of disease. Stem cells have emerged as a promising cell source to address these challenges. Embryonic stem cells (ESCs) and adult

mesenchymal stem cells (MSCs) are the main types of stem cells. Embryonic stem cells have a broaden differentiation spectrum because they can generate all cell types of the three germ layers, ectoderm, mesoderm and endoderm. However, many factors limit their application to human cell therapy. These factors include ethical concerns, immunological incompatibilities and the potential for malignant growth. For these reasons, MSCs are currently the type of choice for therapeutic application **(Sundelacruz and Kaplan, 2009).**

Several studies have suggested that stem cells may protect AKI experimental models from cisplatin, glycerol and ischemia-reperfusion injury **(Herrera et al., 2004, Morigi et al., 2004 and Duffield et al., 2005)**. The mechanism by which bone marrow derived mesenchymal stem cells (BMSCs) functionally share to renal tubular regeneration in AKI is a matter of debate. These experimental models are characterized by extensive necrosis of both proximal and distal tubules that may favor BMSCs migration into these areas. Some studies have reported that the injected BMSCs showed engraftment to the kidney and directly populate the injured renal tubule **(Herrera et al., 2007).** Other studies have found only transient evidence of injected BMSCs in the renal vasculature and no evidence for direct BMSCs incorporation into the tubules during the repair processes suggesting paracrine effects **(Lin et al., 2005)**. Interestingly, the BMSCs conditioned medium (CM), the supernatant of the cultured BMSCS containing growth factors and suspended in DMEM without FBS, has been found to minimize cisplatin-induced renal injury, improved both renal function and structure supporting the hypothesis of paracrine effect **(Bi et al., 2007).**

Aim of the work

This study was undertaken to evaluate the capability of bone marrow-derived MSCs versus its conditioned medium in minimizing the cisplatin induced acute renal injury.

REVIEW OF LITERATURE

I-Cisplatin and acute kidney injury (AKI):

The initial observation of **Rosenberg et al., (1965)** that certain products of electrolysis of platinum electrodes were able to inhibit cell division in *Escherichia Coli*. This event created much interest in the possible use of these products in cancer therapy. Cisplatin was identified as one of these agents.

Kociba and Sleight, (1971) were the first to report cisplatin nephrotoxicity in animal studies. They demonstrated histopathological changes of acute tubular necrosis along with azotemia in male rat study.

Leh and Wolf, (1976) reported that the physico-chemical properties of cisplatin resulted in many problems with its intra-venous administration. Cisplatin had inherent aqueous solubility about $1mg/ml^{-1}$ and therapeutic doses were in the range of 50-100 mg $/m^2$ surface area. This needs intra-venous injection or infusion of minimum 80-180 ml of solution to give the required dose. Many risk factors for cisplatin nephrotoxicity had been identified from many animal studies and human clinical trials. Nephrotoxicity increased with the dose, frequency of administration and cumulative dose of cisplatin **(Madias and Harrington, 1978).**

Patton et al., (1978) used ultra-filtration techniques in man to follow free plasma levels of platinum species. They

reported that these levels became ten times lower after administration of 100 mg cisplatin /m^2 surface area over 6 hours infusion, as compared to plasma levels after bolus injection (within 15 minutes). In addition the total platinum excreted in the urine within 24 hours was about 50% greater than the amount excreted during the same period after bolus injection. This results explained that the route of cisplatin administration has a significant effect on pharmacokinetics behavior.

De conti et al., (1978) studied the *in vivo* platinum clearance rates after intravenous administration of radio-labeled cisplatin to humans. The data indicated biphasic mode of plasma clearance of the labeled platinum, with initial half-life of 25-49 minutes and secondary phase of 58-73 hours. **Gomerly et al., (1979)** added that another slower tertiary phase was present following one hour intravenous infusion of cisplatin at dose of 70 mg/m^2surface area in 8 patients. It was based on plasma platinum determination after three weeks.

Long and Repta, (1981) stated that there were many problems associated with the use of cisplatin as anti-cancer drug. In addition to its toxic manifestations, the low aqueous solubility and nucleophilic substitution reactions resulted in difficulty in development of acceptable methods for cisplatin administration. They reported that cisplatin was highly reactive drug used *in vivo* in human studies. The aqueous solutions of cisplatin degraded via nucleophilic displacement of chloride ligands by water. There were many reactions between cisplatin and naturally occurring biomolecules such

as histidine and methionine. The physico-chemical properties of cisplatin presented many problems in formulations of stable dosage forms. The cisplatin instability in aqueous media required that solutions prepared for intravenous injection must contain added chloride ions. The solubility of cisplatin became better (less than 5% degradation) for up to 24 hours in presence of little concentration of sodium chloride (0.45 %).

Safirstein et al., (1984) stated that cisplatin concentration within the kidney exceeded the blood concentration, suggesting active accumulation of the drug in the kidney cells using a rat model. Also high peak plasma free platinum concentration had been correlated with nephrotoxicity after cisplatin administration to 22 cancer patients at dose of 50-140 mg/m^2 surface area by 2 hour infusion **(Reece et al., 1987)**.

A previous study in patients with metastatic testicular cancer revealed that glomerular filtration rate (GFR) and plasma magnesium concentration decreased after cisplatin dose higher than 50 mg/m^2 body surface area. However these parameters were unchanged if the dose used was below $20mg/m^2$ **(Hartmann et al., 1999).**

Cisplatin (cis-diamminedichloroplatinum, CDDP) is an antineoplastic drug. It was used in treatment of many solid-organ cancers including those of head, neck, lung, testis, ovary and breast. While its side effects included ototoxicity, gastrotoxicity, myelosuppression and allergic reactions, the main dose-limiting side effect was nephrotoxicity.