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Regenerative potential of bone marrow mesenchymal stem cells on cadmium chloride-induced hepato-renal injury and testicular dysfunction in sprague dawley rats

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ABSTRACT

The effect of bone marrow-derived mesenchymal stem cells on cadmium-induced liver and kidney damage was studied in Sprague Dawley rats. The study employed three animal groups: Group 1 served as control animals; Group 2 rats were dosed intra-peritoneally with 2 mg of cadmium chloride per kg body weight, and Group 3 rats were again dosed with a single intraperitoneal injection of 2 mg of cadmium chloride per kg body weight two doses of 10^6 cells each intravenously. Finally, the animals were killed using halothane inhalation anesthesia. Semen analysis (total sperm count, viability, motility, and % of normal sperm), biochemical estimations (serum total protein, uric acid, creatinine, levels of enzymes ALT, AST, and ALP, and levels of hormones LH, FSH, Inhibin, and testosterone), and histopathological analysis of liver and kidney tissue sections (using hematoxylene and eosin stains) were conducted. The results showed that when compared to controls, cadmium exposure drastically decreased total sperm count, viability, motility, and % of normal sperm, decreased serum total protein, increased serum uric acid and creatinine levels, increased levels of ALT, AST, and ALP enzymes, decreased levels of testosterone and inhibin, increased levels of LH and FSH, and caused significant histopathological abnormalities in both kidney and liver tissues. Treatment with stem cells ameliorated the effects of cadmium-induced toxicity significantly ($p < 0.05$) of the histopathological and biochemical parameters. In conclusion, the study reinforces previous findings that bone marrow mesenchymal stem cells can ameliorate the toxic effects of cadmium chloride and may be used as a potential therapeutic strategy for cadmium-induced adverse effects.

1. Introduction

First discovered in Germany in 1817, cadmium is a bluish white, malleable metal found in zinc ores (OSHA, n.d.). The first known use of cadmium was in the production of color pigments, including yellow and red for dyeing purposes. Since then, cadmium has found numerous applications in electroplating, as components in nickel-cadmium rechargeable batteries, in nuclear reactors as a neutron absorber, and in solar cells. North America is the fourth largest producer of cadmium after China, South Korea, and Japan, and over 300,000 workers are exposed to this heavy metal in the US every day (OSHA, n.d.).

Cadmium is produced during the incineration of waste, oil and coal as well and has a extended half-life (about 20–30 years in human beings), making it accumulate in biological systems for long periods of time (Skolarczyk et al., 1970). Humans are exposed to cadmium mainly through food and industrial/occupational exposure. Foods such as grains, fish, vegetables and fruits can be contaminated with this element and the levels of accumulation depend on the location of cultivation. It has been determined that 25% of human exposure to cadmium in the US is through consumption of potatoes (Skolarczyk et al., 1970). Fish can contain up to 0.02 mg/kg of this metal and this is really concerning as the World Health Organization sets the tolerable and safe limit for cadmium intake in adults as only about 0.4–0.5 mg/week. In addition to food sources, cigarette smoking is another source of cadmium and is associated with diabetic complications, hypertension, and cancer (Skolarczyk et al., 1970; Yang and Shu, 2015).

Cadmium accumulation occurs primarily in the soft tissues like the liver, kidney and testes (Yang and Shu, 2015; Rani et al., 2013). The

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primary toxic form of cadmium is the Cd2 + ion and as such, this ion has no physiological role in the human body. When the body is exposed to Cd2 + , the ion accumulates in the soft tissues where it competes with essential ions for entry into cells (Thévenod and Lee, 2013).

The mechanisms involved in cadmium induced toxicity to the liver and kidneys have been well elucidated. In the liver, cadmium exposure leads both to direct tissue injury and indirect adverse effects through inflammatory responses (Rikans and Yamano, 2000). Direct tissue injury occurs when Cd2 + ions enter the cell and interact with, and inactivate the sulfhydryl groups on molecules (like glutathione) associated with the mitochondria. This inactivation leads to oxidative stress, dysregulation of mitochondrial membrane permeability and loss of mitochondrial function (Rani et al., 2013). The resultant ischemia is believed to lead to damage of epithelial cells and hepatocellular injury (Rikans and Yamano, 2000). Indirectly, cadmium ions activate Kupffer cells and inflammatory mediators in the hepatocytes, and initiate neutrophil infiltration (Rikans and Yamano, 2000; Rani et al., 2013). Similar mechanisms of action for cadmium toxicity have also been observed in kidney epithelial cells (Yang and Shu, 2015). Here, a number of cellular membrane transporters including metallothioneins, zinc, and calcium transporters are involved in transporting cadmium ions into the cells, where they induce toxic effects leading to cell injury and apoptosis (Thévenod and Lee, 2013).

Another organ that is disastrously affected by cadmium exposure is the testes (Elbaghdady et al., 2018; Marettová et al., 2015). Testicular damage occurs even at non-toxic levels of cadmium exposure and is manifested as low sperm count, poor semen and sperm quality, azoospermia, and infertility in rats and other animal models (Elbaghdady et al., 2018).

In light of the continuing human exposure to, and the extent of damage caused by heavy metals like cadmium, there is a rising need for strategies to ameliorate cadmium-induced toxicity. Over the years, several studies have examined a number of natural and synthetic remedies and recently, mesenchymal stem cells (MSCs) have been found to have great potential as a means to reverse cadmium-induced effects. MSCs have been found to help repair and regenerate injured testicular, hepatic, and renal cells following acute exposure to cadmium through their quick replicative and multi-potent properties (Lei et al., 2015; Elbaghdady et al., 2018).

Over the years, there has been much speculation over the mechanisms by which MSCs bring about tissue repair. While it was initially suggested that tissue repair was performed by trans-differentiation or cell fusion of the transplanted MSCs with host cells. However, both these mechanisms have been found inefficient as much of the grafted MSCs are not accepted or are destroyed during the process (low engraftment rate) (Gnecchi et al., 2016). It is now believed that the primary mechanism of tissue repair brought about by MSCs is via soluble factors secreted by these cells that function in a paracrine manner to improve tissue function following injury (Gnecchi et al., 2016). Further, Tomasoni et al. (2013) demonstrated that MSCs delivered mRNAs to injured epithelial cells through an exsosome-mediated mRNA transfer, suggesting that this could be a mechanism by which MSCs mediate tissue repair.

Multipotency is the ability of cells to differentiate into all the different cell types within a particular lineage (Jaenisch and Young, 2008; Krampera et al., 2007). Further, multipotent stem cells are undifferentiated cells that can self-renew for extended periods of time and differentiate into different cell types with their own specific functions (Murphy et al., 2013; Mundra et al., 2012).

It is believed that the multipotent nature of MSCs allow them to maintain optimum tissue physiology through the repair and replacement of injured cells and tissues exposed to cadmium (Hsaio et al., 2015). In a parallel study, Yang et al. (2015) determined that the transplantation of rat adipocyte-derived MSCs into D-galactose treated aging rats could result in amelioration of testicular dysfunction through the differentiation of these MSCs into 3-β hydroxysteroid dehydrogenase-positive Leydig-like cells. Wang et al. (2017) also found that MSCs could prevent cadmium-mediated mitochondrial injury. These findings show that MSCs have the capability to both directly prevent the toxic effects of cadmium, in addition to repairing damaged tissues.

In order to reinforce and validate these findings and to determine the ameliorating effects of MSCs on cadmium-induced tissue injury, the present study was performed. The present study examined the nature of cadmium toxicity through the analysis of semen, histopathological study of liver and kidney tissues, and biochemical analysis of liver enzymes and reproductive hormones, and compared these effects following supplementation with MSCs in Sprague Dawley rats. The study hypothesizes that acute exposure to cadmium chloride induces hepatic, renal, and testicular injury and dysfunction, and MSCs ameliorate cadmium-induced toxicity at the tissue and biochemical level.

In conclusion, Humans and animals living in areas containing cadmium (contaminated water and environmental pollution) are exposed to low doses of cadmium over a long period of time. In other words, they are exposed to chronic toxicity. Acute toxicity, though rare, can also happen if the dose of cadmium is sufficiently high. To simulate the consequences of such acute toxicity (the disease and its symptoms), the present study exposed the experimental animals to a high dose of CdCl2 for a short period of time (acute exposure). The results obtained from the study are expected to show the efficacy of MSCs in alleviating these consequences, and if successful, become a potential therapeutic strategy for heavy metal toxicity.

2. Materials and methods

2.1. Animals

Thirty male Sprague Dawley rats aged 10–12 weeks and weighing between 150 and 170 g were used in this study. The animals were procured from the Animal House of Nile Center for Experimental Research, Mansoura, Egypt, and were allowed to acclimatize for 2 weeks before the initiation of the experimental study. The rats were randomly divided into three groups (each group consisting of 10 healthy animals) and the following groups were assayed

Group 1 (Control group): The group did not receive any treatment and served as controls for all experiments.

Group 2 (Cadmium group): Each animal in the group received a single intraperitoneal dose of cadmium chloride at 2 mg/kg body weight.

Group 3 (Cadmium + MSC group): Each animal in this group received a single intraperitoneal dose of cadmium chloride at 2 mg/kg body weight. The rats were exposed to CdCl2 for 4 weeks after single intra-peritonial dose before analysis.

Following the induction of hepato-renal injury in these rats, the rats received two doses of mesenchymal stem cells (MSCs) (2×10^6 cells/ rat) through intravenous injection into the portal vein. The two doses were separated by a period of 1 week and each dose (10^6 cells/ rat) was suspended in 0.2 ml Dulbecco's modified Eagle medium (DMEM). Finally, only 30 rats were used (divided into 3 groups of 10 animals). However, the analyses were done in triplicate to ensure accuracy and precision of results.

A dose of 2 mg/kg of cadmium is a realistic dose experimentally found to induce hepato-renal and testicular damage (Adamkovičová et al., 2010).

2.2. Isolation and preparation of mesenchymal stem cells (MSCs)

The protocol provided by Elbaghdady, Alwaili, & El-Demerdash (2018) was used for the isolation and preparation of MSCs.

The rats were sacrificed two weeks following the final treatment with MSCs. This time was provided to allow the MSCs to have a chance to repair the injury to organs caused by cadmium exposure. Briefly, rats