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Original article

Comparative study on the effect of low intensity laser and growth factors on stem cells used in experimentally-induced liver fibrosis in mice

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ABSTRACT

Background and study aims: The therapeutic effects of human umbilical cord-derived mesenchymal stem cells (UC-MSCs) exposed to diode laser and/or hepatocyte growth factor (HGF) were compared in mice with experimental liver fibrosis induced by carbon tetra chloride (CCl_4).

Material and methods: Animal model of liver cirrhosis was induced by intraperitoneal injection of CCl_4 in a dose of 0.4 ml/kg, twice a week for 6 weeks. UC-MSCs were obtained from normal full term placentas and were exposed to diode laser and/or HGF. Before treatment, UC-MSCs were labelled with red fluorescent PKH26. Fifty four male mice weighing 25–35 g were randomly divided into four groups control, stem cells, CCl_4, and treated groups. After the experimental period, body and liver weights were recorded, and the liver specimens were processed for histological examination using haematoxylin and eosin, Periodic Acid-Schiff (PAS), and Masson's Trichrome staining (MT).

Results: Results showed that administration of UC-MSCs stimulated by diode laser and/or HGF improved body and liver weights, reduced vascular dilatation and congestion, reduced mononuclear cellular infiltration, reduced hepatocyte vacuolation, eosinophilia, and pyknosis. Furthermore, periportal fibrosis was minimized and PAS reaction was increased. These effects were maximum when UC-MSCs were exposed to both diode laser and HGF.

Conclusion: UC-MSCs stimulated by both diode laser and HGF proved to be an effective therapeutic option in experimental liver fibrosis induced by CCl_4 in mice.

Introduction

Liver diseases constitute a major health problem in many parts of the world [1]. The endpoint of the progressive damage to the liver is loss of 80–90% of hepatic functional capacity with 70–95% mortality from hepatic failure [2]. Fibrogenesis is characterized by excessive accumulation of extracellular matrix (ECM) components. The net accumulation of the ECM connective tissue results from enhanced synthesis, or diminished breakdown of the matrix, or both. Collagens, predominately types I and III, are the major fibrous proteins in ECM and their synthesis increases in the liver exposed to carbon tetrachloride (CCl_4) [3].

While transplantation is currently an accepted therapy for liver disease, there remain many challenges as shortage of donors and the harshness of the invasive procedures [4,5]. The use of stem cells for future tissue engineering and regenerative medicine to replace conventional therapeutic methods has been the subject of growing interest in different areas. These cells have self-renewing properties and are able to differentiate into one or many different specialized cell types [6]. Stem cell research has expanded well due to their usefulness in regenerative therapies for improving the life of patients suffering from various genetical and neurological diseases [7].

Mesenchymal stem cells (MSCs) can be found in various locations in the body, including the bone marrow [8], adipose tissue [9], and peripheral blood [10]. MSCs can differentiate into several types of mature cells, including neurons, adipocytes, cartilage, skeletal muscle, hepatocytes, and cardiomyocytes, under appropriate conditions [9,11].

Umbilical cord derived mesenchymal stem cells (UC-MSCs) isolated from human placenta can differentiate into hepatocytes in the normal liver and in some pathologic environments. Stem cells are undifferentiated cells that divide to replenish dying cells and regenerate damaged tissues. [12]. Bone marrow derived mesenchymal stem cells (BM-MSCs) have the capacity to differentiate into hepatocyte-like cells, as well as their ability to reduce fibrogenesis [13–15].

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UC-MSCs ameliorated liver fibrosis in rats with carbon tetrachloride (CCl_4)-induced cirrhosis. However, these results showed some variation across different animal models, CCl_4 treatment protocols, and in vitro conditions [14,15].

Although BM-MSCs represent an attractive therapeutic alternative for treating degenerative diseases, their use is limited by several factors, including low cell yields from donor BM, dependence on donor age, limitations to autologous use, and difficulty in recruiting donors [13,16–18].

UC-MSCs, which have received much research attention, exhibit characteristics similar to those of BM-MSCs, but they bear several advantages [19]. UC-MSCs display multi-lineage differentiation potential, and they are free of ethical concerns, easily accessible, abundant, and strongly immunosuppressive [17,20,21]. Minimal criteria for defining UC-MSCs are (i) foetal origin; (ii) generation of fibroblast colony-forming units; (iii) specific patterns of surface antigen expression; and (iv) potential to differentiate into one or more lineages [22]. UC-MSCs have the potential to differentiate in vitro into hepatocyte-like cells and insulin-positive cells, as well as mesodermal lineages [22–24].

There is increasing evidence that the age of the donor tissue affects several properties of mesenchymal stem cells and this fact cause decreased repair capacity and increased susceptibility to degenerative diseases [18,25].Wharton's jelly derived MSC, in their short, prenatal life do not have proaging factors.

In comparison to MSC from adult tissues, UC-MSCs at such an early embryonic state retain telomere at highest possible length, which protects them from premature loss of viability [26].

Laser phototherapy has been used to treat pathological tissue conditions, to control inflammatory processes and also to promote tissue healing. The mechanism by which low intensity lasers induce biomodulation of cell activity has been well described by Karu [27]. Laser irradiation is postulated to intensify the formation of a transmembrane electromechanical proton gradient in mitochondria. Thus, the efficiency of the proton-motive force (pmf) is increased and more calcium is released into the cytoplasm from the mitochondria. At low laser doses, this additional calcium transported into the cytoplasm triggers mitosis and enhances cell proliferation. [6].

Hepatocyte growth factor (HGF) is a polypeptide that affect a number of cellular processes such as proliferation and differentiation both *in vivo* and in vitro [2]. It has potent cytoprotective action on hepatocytes, besides enhancing liver regeneration. HGF also stimulates the migration and proliferation of activated hepatic stem cells into the liver parenchyma, where the cells differentiate into mature hepatocytes [29].

The aim of the present study was to compare the therapeutic effects of human umbilical cord derived mesenchymal stem cells (UC-MSCs) exposed to diode laser and/or HGF in mice with experimental liver fibrosis induced by carbon tetra chloride (CCl_4).

Material and methods

Drugs and chemicals

Carbon tetrachloride (CCl_4): CCl_4 and PKH26 Red Fluorescent Cell Linker kit were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hepatocyte growth factor was purchased from Koma Biotech, Seoul, Korea.

Animals

Animals: fifty-four male mice weighing 25–35 g, 2–3 months old, were obtained from the Holding Company for Biological Products & Vaccines (VACSERA), Cairo, Egypt. The animals were divided into 8 groups each with 6 animals. They were bred and maintained on standard laboratory rodent diet. The animals were maintained at 12-h light/dark cycles at constant temperature (20 ± 1 °C) and humidity (50 ± 5%). The study complied with the Guide for the Care and Use of Laboratory Animals [30].

Experimental design

Animals were randomly divided and treated in the following way:

Group I – Control Group: subdivided into:

Subgroup I-a: Negative control group: six mice; received no treatment.
Subgroup I-b: Vehicle group: six mice; each received intraperitoneal (i.p.) injection of olive oil twice a week for 6 weeks, at the same volume as CCl_4-treated mice.

Group II – Stem cells group: subdivided into:

Subgroup II-a: 6 mice; each received UC-MSCs treatment without exposure neither to diode laser nor HGF
Subgroup II-b: 6 mice; each received UC-MSCs irradiated with diode laser.
Subgroup II-c: 6 mice; each received UC-MSCs exposed to HGF.

Group III – CCl_4-treated group: 6 mice; each received i.p. CCl_4.
Group IV – Experimental group: subdivided into:

Subgroup IV-a: 6 mice; each received CCl_4 for 6 weeks followed by UC-MSCs irradiated with diode laser.
Subgroup IV-b: 6 mice; each received CCl_4 for 6 weeks followed by UC-MSCs exposed to HGF.
Subgroup IV-c: 6 mice; each received CCl_4 for 6 weeks followed by UC-MSCs exposed to both diode laser irradiation and HGF.

Induction of liver fibrosis

Mice of groups III and IV were given CCl_4 intraperitoneally (i.p.) at a dose of 0.4 ml/kg, dissolved in olive oil, twice a week for 6 weeks [31]. In group IV, UC-MSCs were injected i.v. after the end CCl_4 treatment.

Isolation and cultivation of UC-MSCs from Wharton jelly

The collection of placenta samples was done in Cairo University Hospitals and their subsequent utilization for research purposes was approved by the Institutional Review Board of Faculty of Medicine, Cairo University. All participating women provided written, informed consent prior to the collection of samples. Placentas were collected from women who were free of medical, obstetrical, and surgical complications and who delivered at term (37 gestational weeks). Briefly, the umbilical cords (UCs) were collected in a transfer medium of phosphate-buffered saline (PBS) and 50 IU heparin, and were maintained at 4 °C until processing, which was within 24 h of collection. UCs were washed three times in PBS, and the umbilical veins were rinsed with PBS to remove contaminating red blood cells. UCs were cut into 1 cm segments, and UC arteries, veins and amnion were removed. The gelatinous tissue was excised, and minced into 0.5–1 mm^3 pieces. Equal volumes of PBS were used to swell the tissue pieces with constant shaking at 4 °C for 48 h, and the tissue pieces were subjected to 5 freeze–thaw cycles (−80–37 °C). Samples were ruptured by high-speed dispersion on ice for 10 min, and homogenized with a glass homogenizer on ice. Tissue homogenates were centrifuged at 10,000*g* for 30 min

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