Role of Human Wharton's Jelly Derived Mesenchymal Stem Cells (WJ-MSCs) for Rescue of D-Galactosamine Induced Acute Liver Injury in Mice

Raghu Ramanathan [*], Secunda Rupert [*], Sakthivel Selvaraj [*], Jeswanth Satyanesan [*], Rosy Vennila [*], Surendran Rajagopal [†]

[*]Centre for Advanced Research, Stem Cell Research Centre, Govt. Stanley Medical College and Hospital, Chennai, Tamilnadu, India and [†]Director, Hepato-Pancreato-Biliary Centre for Surgery & Transplantation, MIOT International, Chennai, Tamilnadu, India

Background/Aim: **Mesenchymal stem cells (MSCs) are multipotent precursor cells having self-renewal ability making them a candidate for use in regenerative medicine. Acute liver injury results in sudden loss of hepatic function leading to organ failure. Liver transplantation is often required to salvage patients with acute liver failure. Due to shortage of organs, identification of alternate method is the need of the hour. In view of this, an attempt has been made to check the regenerative ability of WJ-MSCs (wharton's jelly derived MSC) in mice models for acute liver injury.** ***Methods:*** **Swiss albino mice weighing 25 ± 5 g were used in this study. The control mice (Group I), was given saline. Group II mice received D-Galactosamine (D-GalN—800 mg/kg; i.p). Group III mice similar with Group II, received WJ-MSCs (5×10^5 cells/0.5 ml DMEM) through tail vein, 24 h after D-GalN administration and Group IV mice received MSC alone.** ***Results:*** **Parameters, indicative of hepatotoxicity and oxidative stress were analyzed. A two-fold elevation in the marker enzymes of liver toxicity such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (SAP), and total serum bilirubin (TBIL) confirms hepatocellular injury, while a greater than four-fold increase in malondialdehyde (MDA) formation, along with around 40% fall in superoxide-dis-mutase (SOD) activity was indicative of oxidative stress and loss of hepatocellular membrane integrity induced by D-GalN. The above biochemical and pathological changes were significantly restored in mice that received WJ-MSCs indicating hepatoprotective and probable regenerative property.** ***Conclusion:*** **The present study showed that WJ-MSC treatment is able to rescue/ameliorate the hepatotoxicity induced by D-GalN in mice. (J Clin Exp Hepatol 2017;7:205–214)**

The acute liver failure (ALF) is a clinical condition which results in both fulminant and subfulminant hepatic failure. There are many causes of ALF such as viral infections, drugs and chemicals-induced toxic injury, alcohol abuse, autoimmune disorders and genetic disorders leading to liver failure.[1] No specific medical treatment is presently available. Liver disease is an important cause of morbidity as well as mortality worldwide.[2] Liver transplantation is the only effective therapy available till date for all chronic end-stage liver diseases and many cases of ALF. Identifying an alternate remedy is the need of the hour, considering the shortage of available donor organs, high costs and requirement for lifelong immunosuppressive drugs following liver transplantation.

D-Galactosamine (D-GalN) is a well-known hepatotoxicant, which causes severe hepatocellular necrosis and other clinical features similar to that of human viral hepatitis in experimental animals. D-GalN is a specific hepatotoxic agent that inhibits macromolecule synthesis such as RNA and proteins resulting in organ dysfunction. Some studies have shown that the reactive oxygen species (ROS) generated by metabolism of D-GalN resulted in hepatotoxicity.[3] A recent study by Pushpavalli et al. (2010),[4] concludes that oxidative stress and inflammatory response are the two main factors for D-GalN-induced acute liver injury. Rodents administered with D-GalN show remarkable sensitization to lipopolysaccharide (LPS), and this D-GalN/LPS treatment in animals has been recognized as one of the promising model to study the clinical conditions of ALF in vivo.[5] D-GalN is known to selectively block transcription and indirectly influence hepatic RNA and protein synthesis as a consequence of endotoxin toxicity.[6] D-GalN mainly causes liver injury via the generation of free radicals and depletion of uridine triphosphate (UTP) nucleotides leading to progressive damage of cellular membranes and in higher doses, it is known to cause hepatic necrosis.[7,8]

Stem cell based cell therapy has now gained interest as an alternative option for treating ALF.[9,10] Specifically mesenchymal stem cells (MSCs) are being explored as an

Keywords: D-galactosamine, WJ-MSCs, oxidative stress, hepatotoxicity, cytogenetics
Received: 25.10.2016; *Accepted:* 14.03.2017; *Available online:* 1 April 2017
Address for correspondence: Rosy Vennila, Centre for Advanced Research, Stem Cell Research Centre, Govt. Stanley Medical College and Hospital, Chennai, Tamilnadu, India. Tel.: +91 044 25280032.
E-mail: stemcellstanley@gmail.com
Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; MSCs: mesenchymal stem cells
http://dx.doi.org/10.1016/j.jceh.2017.03.010

effective therapeutic tool in various types of experimental liver injuries in animals.[10,11] MSCs can be isolated from various tissues, including bone marrow, muscle, tooth, amniotic fluid, placenta, adipose tissue.[12,13] MSCs are also known to have regenerative and anti-inflammatory properties[5] which make them suitable for use in regenerative medicine. Currently MSCs are being explored in inflammation-related diseases, such as acute lung injury, neuropathic pain, acute respiratory distress syndrome, and renal injury due to its immunomodulatory effects along with interleukins which play a major role against the above diseases.[14] Recently it is found that WJ-MSCs has the ability to escape immune surveillance due to the absence of the antigen major histocompatibility complex II (MHC-II) and the co-stimulatory surface antigens CD40, CD80, and CD86.[15–17] These findings suggest that WJ-MSCs has low immunogenicity and might be well tolerated in xeno-transplantation without immunosuppression. Previous studies have also reported that transplantation of human MSCs without immunosuppression produces beneficial effect in animal models of Alzheimer's disease, Parkinson, traumatic brain injury, and stroke.[18–22] So this interested us to use WJ-MSCs in our present study to analyze whether the MSCs derived from wharton's jelly is able to rescue the hepatotoxicity induced by D-GalN in acute liver injury in mice model.

MATERIALS AND METHODS

Isolation and Culture of WJ-MSC

Informed consent was obtained from mother prior to delivery for collecting the umbilical cord aseptically, sectioned and transported from Govt. Raja Sir Ramaswamy Mudaliar (RSRM) Lying-in Hospital, Chennai to the Stem cell research lab in Govt. Stanley Hospital, Chennai, Tamilnadu, India. The samples were transported within 15 min and processed immediately without further delay. MSCs were cultured by our previously standardized protocol.[23] Explants were removed from the culture flask on day 7 and the medium was renewed every 48 h and sub cultured on reaching 70–80% confluence.

Detection of Surface Markers of WJ-MSCs Using Flow Cytometry

Cell surface marker expressions were determined on all groups of MSCs, cultured in PL from P1 to P5. The primary antibodies used were fluorescent conjugated anti-human CD90-FITC, CD73-APC, CD105-PerCp-Cy5.5 (mesenchymal lineage), CD14-FITC, CD34-PE and CD45-APC-CY7 (hematopoietic lineage). Flow cytometry was performed using fluorescence activated cell sorter (BD Facs Aria II) and the results were analyzed with DIVA software.

G-banded Karyotyping

Standard cytogenetic procedures were followed for analysis of WJ samples. When the culture reached confluence of 80%, Colcemid (10 mg/ml) was added to each flask to a final dilution of 0.1 μg/ml and then incubated at 37 °C for 30 min. Changes in cell morphology were monitored using an inverted microscope. The MSCs were detached using 0.25% trypsin-EDTA. After 3 min of monitored detachment, few drops of FBS were added and the cells were transferred to a centrifuge tube containing medium. Samples were centrifuged at 400 × *g* for 10 min. For the hypotonic treatment, 10 ml of 0.075 M KCl was slowly and carefully added, followed by incubation at 37 °C for 30 min and fixation using methanol: acetic acid (3:1) solution. To improve the quality of metaphases, the samples were washed twice with methanol: acetic acid (3:1). Prior to slide preparation, slides were cleaned and kept in cold, immersed in water. Samples were dropped on to the surface of the slide from a distance to form a uniform blast. In order to obtain G-bands, the slides were baked at 80 °C for one hour and then aged at 37 °C for at least 16 h. Then, they were immersed in Trypsin solution (0.002 g/mL) for 5 s, washed in Sorenson's buffer and finally quickly rinsed in distilled water. The staining procedure was carried out using Giemsa (1: 20) producing Trypsin and Giemsa (GTG) bands. The band quality was evaluated under the microscope (magnification;100X) and the Trypsin and staining times were adjusted to produce clear well stained bands. Twenty five metaphases were analyzed per sample for karyotyping and the karyogram was performed using Applied Spectral Imaging (ASI) software.

Chemicals

Galactosamine (D-GalN), 1,1,3,3-tetramethoxypropane malondialdehyde (MDA), thiobarbituric acid, DMEM, Trypsin, antibiotics, Epinephrine, Bicinchoninic acid, Copper (II) Sulfate Pentahydrate, Bovine serum albumin (BSA), Glacial acetic acid and Giemsa were purchased from M/s Sigma–Aldrich Chemicals (St. Louis, Missouri, USA). Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS) and Karyomax—Colcemid were purchased from Gibco life technology, USA. The primary antibodies such as CD90-FITC, CD73-APC, CD105-PerCp-Cy5.5, CD14-FITC, CD34-PE and CD45-APC-CY7 for flow cytometer were purchased from M/s BD Biosciences, Franklin Lakes, NJ, USA. All the other chemicals used for various assay procedures were purchased locally and were analytical grade.

Animals

Swiss albino mice, weighing 25 ± 5 g procured from Institutional Animal House facility of Madras Medical College were used in this study. Animals were maintained