Reversal of Hepatic Fibrosis By Stem Cell Transplantation in Rats

Thesis

Submitted for partial fulfillment of

By

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Abstract

A model for liver fibrosis was prepared using CCl_4 injected into rats. Human cord blood-derived mononuclear cells (MNCs) were cultured. $CD34^+$ cells were isolated from MNCs. A candidate $CD34^+$ stem cell population were separated from $CD34^+$ cells by adherence to tissue culture plastic. Cells were cultured with and without hepatic differentiation medium. Rats were divided into groups and injected with differentiated and undifferentiated cells through intrahepatic and intravenous routes aiming to evaluate the ability of these cells to reverse hepatic fibrosis.

The results of the present study show a significant elevation in serum albumin after administration of stem cells compared to the CCl_4 group. IV. differentiated cells was significantly lower than the other groups that received stem cells. As regards liver enzyme, ALT, there was a significant decrease of its level compared to the CCl_4 group. However, it was still significantly higher than control with no significant difference between the groups that received stem cells. Histopathological examination of liver tissue showed that stem cells have a significant antifibrotic effect with no significant difference between the groups that received stem cells. Concerning gene expression, the collagen gene (rat) was highly expressed in the CCl_4 group whereas its expression was significantly decreased after administration of stem cells with no significant difference between the groups that received stem cells.

The human albumin and matrix metalloproteinase (MMP2) genes were expressed in liver tissues in the groups that received stem cells. Highest expression was in the group that received undifferentiated cells IV.

The results of the present work reveal that administration of $CD34^+$ stem cells derived from human cord blood can ameliorate liver fibrosis in rats. The degree of differentiation and route of administration didn't affect liver functions or the degree of fibrosis.

Key words: Stem cells, liver fibrosis, hematopoietic stem cells.

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List of abbreviations

AFP	Alpha-fetoprotein
ALB	Albumin
ALF	Acute liver failure
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BM	Bone marrow
BMSCs	Bone marrow stem cells
CB	Cord blood
CCl_4	Carbon tetrachloride
CD	Cluster of Differentiation
cDNA	Complementary DNA
CFU	Colony forming units
CFU-F	Colony forming unit-fibroblast
CK	Cytokeratin
CTLA4	Cytotoxic T lymphocyte antigen-4
CXCR-4	CXC chemokine receptors-4
DDR_2	Discoidin domain receptor
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
EB	Ethidium Bromide
ECM	Extracellular matrix
EDTA	Ethylene Diamine Tetra Acetate
EGF	Epidermal growth factor
EPCs	Endothelial progenitor cells
ESC	Embryonic stem cells
FACs	Fluorescence activated cell sorting
FAH	Fumaryl acetoacetate hydrolase
FBS	Fetal bovine serum
FGFs	Fibrogenic growth factors
G-CSF	Granulocyte- colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
GTC	Guanidine thiocyanate
HCC	Hepatocellular carcinoma
HC-gp-39	Human cartilage glycoprotein-39
HCV	Hepatitis C virus
HE	Hematoxylin and eosin
HGF	Hepatocyte growth factor

HLA	Human leukocyte antigen
HOCs	Hepatic oval cells
HPRI	Human placental ribonuclease inhibitor
HSCs	Hematopoietic stem cells
IFN-γ	Interferon- γ
IGF-1	Insulin-like growth factor-1
IH.	Intrahepatic
IL	Interleukins
IV.	Intravenous
KDa	Kilodalton
LDL	Low density lipoprotein
LFA	Lymphocyte function-associated antigen 1
MACS	Magnetic cell sorting.
MAPCs	Multipotent adult progenitor cells
MEM	Modified Eagle's medium (minimal essential medium).
MMLV	Moloney murine leukemia virus.
MMPs	Matrix metalloproteinases.
MNCs	Mononuclear cells
MPC	Mesenchymal progenitor cells
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MSCs	Mesenchymal stem cells
MSF	Marrow stromal fibroblasts
NADPH	Nicotinamide adenine dinucleotide phosphate
NASH	Nonalcoholic steatohepatitis
NF-κB	Nuclear factor- Kb
NOD	Nonobese diabetic
NPE	Non-parenchymal epithelial progenitor
OCs	Oval cells
OSM	Oncostatin M
PBC	Primary biliary cirrhosis
PBS	Phosphate buffer salin
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PPAR□	Peroxisome proliferator-activated receptor
RNA	ribonucleic acid
RNAse	Ribonuclease
ROS	Reactive oxygen species

Rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
SCF	Stem cell factor
SCID	Severe combined immunodeficient
SCNT	Somatic cell nuclear transfer
SDF-1	Stromal-derived factor 1
SHPCs	Small hepatocyte-like progenitor cells
SOCS-1	Suppressors of cytokine signaling-1
SREBP-1c	Sterol-regulatory element-binding protein-1c
TAE	Tris-Acetate EDTA
Taq	Thermus aquaticus.
TGF-β1	Transforming growth factor- beta 1
Th	T helper cells
TIMP-1	Tissue inhibitor of metalloproteinas
TIPS	Transjugular intrahepatic protosystemic shunt
TNF-α	Tumor necrosis factor alpha
UCB	Umbilical cord blood
UV	Ultraviolet
VLA	Very late antigen.

Introduction

Liver transplantation is the gold standard treatment for end-stage liver failure and for numerous liver based inborn errors of metabolism. However, organ shortage remains a major limiting factor and alternative solutions are being examined in the liver therapy field. Liver cell transplantation is emerging with heartening success (**Stephenne et al., 2006),** but is still limited by cell viability, modest engraftment and limited tissue availability. Increasing interest is carried to stem cells regarding the recent demonstration of their plasticity (**Verfaillie et al., 2002**).

Several sources of stem cells have been proposed as sources for cell therapy. Embryonic stem cells are the most potent in terms of their differentiation potential but may be tumorigenic when transplanted in vivo, and their use is limited by ethical issues (**Fujikawa et al., 2005**). Adult stem cells may be found in any tissue (**Preston et al., 2003**), but hematopoietic tissue is most accessible. Hematopoietic tissue contains two types of stem cells, the mesenchymal and hematopoietic stem cells. **Abdel Aziz et al.** (**2007**) showed that bone marrow–derived mesenchymal stem cells can ameliorate liver fibrosis in rats. Stem cells in hematopoietic tissue have been used for hematological reconstitution for many years (**Thomas, 2005**). These cells are $CD34^+$ and $CD133^+$ and give rise to all lineages of blood cell differentiation. Thus, they have the advantage that they can be prospectively isolated from hematopoietic tissue in known numbers.

In humans, **Theise et al.** (**2000**) showed that the adult human hematopoietic stem cell population can yield hepatocytes upon instruction by the appropriate environment. **Korbling et al.** (**2002**) showed that

hepatocytes are generated from the bone marrow of recipients of sex-mismatched bone marrow transplants at a high frequency that ranges from 4% to 7%. Moreover, **Ng et al.** (**2003**) found that in human liver allografts, although most of the recipient-derived cells showed macrophage/Kupffer cell differentiation, recipient-derived hepatocytes were also present and constituted 0.62% of all the hepatocytes in the recipient. To examine the mechanisms by which human hematopoietic cells contribute to liver regeneration, the human-to-mouse xenogeneic transplantation model was used.

Several reports have shown that when human cord blood (CB) cells (all cells, $CD34^+$ cells, or $CD45^+$ cells) are injected into mice through either the portal vein or the systemic circulation, they can form human hepatocyte-like cells in the murine liver environment (**Kakinuma et al., 2003)**. However, even when there is massive liver damage, the frequency with which this hepatocytic differentiation occurs is low compared to that reported in human-to-human transplantation studies. This low level of efficiency makes it hard to clarify whether transdifferentiation or cell fusion is the primary mechanism that generates hepatocytes from human hematopoietic cells.

Aim of the work

The present study aimed to clarify the role played by human $CD34^+$ stem cells to ameliorate liver fibrosis in rats. Also, to investigate whether there is an effect of the route of administration as well as the degree of differentiation of the stem cells on the reversal of fibrosis.

Liver Fibrosis

Introduction

In humans, the liver is the second largest organ in the body (the skin being the largest). The liver is responsible for performing more functions than any other organ in the body, including metabolizing the different food elements; filtering and detoxifying (neutralizing) poisons in our blood to remove numerous toxic compounds, producing immune agents to control infection, and regenerating itself when part of it has been damaged. Several times each day, the entire blood supply passes through the liver.

Another important function of the liver is to produce prothrombin and fibrinogen (two blood-clotting factors) and heparin (a glycosaminoglycan sulfuric acid ester that helps prevention of blood from clotting within the circulatory system). The liver also converts sugar into glycogen and stores it. The released glycogen becomes glucose in the blood stream. The liver also synthesizes proteins and cholesterol and converts carbohydrates and proteins into fats and stores them for later use.

Additionally, the liver produces and secretes bile, which is needed for the breakdown and digestion of fatty acids. It also produces plasma proteins and hundreds of enzymes needed for digestion and other body functions. As the liver breaks down proteins, it produces urea, which it synthesizes from carbon dioxide and ammonia.

Essential trace elements, such as iron and copper, as well as vitamins A, D, and B12 are also stored in the liver.