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Review Article

The Role of Mesenchymal Stem Cells in Liver Regeneration

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Abstract:

Background: Inflammation of the liver caused by cholestasis, viral infection, alcohol, autoimmune reactions, toxins, or metabolism will result in a prolonged immune response. As a result, simultaneous inflammation and tissue remodelling occur, resulting in fibrosis and eventually leading to cirrhosis. The main treatment for end-stage liver cirrhosis is liver transplantation. However, it is often not possible for patients to undergo this life-saving procedure. On the other hand, stem cell transplantation may be a potential strategy to prevent disease progression and improve the degree of fibrosis.

Discussion: Inflammation of the liver activates hepatic stellate cells, which are perisinusoidal cells in the Disse cavity that contain vitamin A. Hepatic stellate cells activation results in retinoid storage loss and transformation into myofibroblast-like cells that express α-smooth muscle action (α-SMA) and produce collagen which plays a major role in fibrosis. Liver regeneration due to chronic liver damage is played by mesenchymal cells through the mesenchymal-epithelial or epithelial-mesenchymal transition (MET/EMT) process. Administration by the intrahepatic route is thought to be the ideal route because fewer cells are lost in the circulation and more mesenchymal stem cells differentiates into hepatocytes in the damaged liver area. However, intrasplenic route maybe an alternative with easier administration technique. There are special considerations regarding the risks, including the risk of carcinogenesis and viral transmission.

Conclusion: Mesenchymal stem cells transplantation may be a potential therapeutic strategy for patients with end stage liver disease in the future. However, future research is needed regarding the risk of carcinogenesis and viral transmission following the procedure.

Keywords: mesenchymal stem cells, liver regeneration, fibrosis

Liver Fibrosis and Regeneration

Inflammation of the liver caused by cholestasis, viral infection, alcohol, autoimmune reactions, toxins, or metabolism will result in a prolonged immune response. As a result, simultaneous inflammation and tissue remodelling occur, resulting in fibrosis and eventually leading to cirrhosis. Inflammation activates hepatic stellate cells, which are perisinusoidal cells in the Disse cavity which contain vitamin A.[1] Hepatic stellate cells activation results in retinoid storage loss and transformation into myofibroblast-like cells that express α-smooth muscle action (α-SMA) and produce collagen which plays a major role in fibrosis. In addition, there was an increase in tissue inhibitor of metalloproteinase-1 (TIMP-1) which inhibited extracellular matrix resolution thereby increasing collagen deposition.[2]

Matrix deposition due to liver damage is a transient process, thus optimal recovery will eliminate the matrix.[3] Fibrosis occurs when a substantial amount of collagen matrix accumulates, producing scars and distorting the architecture and consistency of the liver; thus, it is known as cirrhosis. If the injury or inflammatory response persists, the parenchyma will be replaced by a connective tissue matrix containing collagen and elastin fibers that bond progressively, leaving the scar difficult to break down by enzymes.[4]

Regeneration and fibrosis are linked by a cascade that is triggered by an injury and then branches out depending on the severity or duration of the damage. This cascade involves the interaction of epithelial, mesenchymal, endothelial, and immune cells.[3] During the liver damage, dead hepatocytes or foreign antigens are recognized by Kupffer cells. Recruited monocytes are activated into macrophages, which then produce tumor growth factor-β (TGF-β). TGF-β induces transcription of collagen types I and III through the Smad signalling pathway.[5] Furthermore, these cells will release pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), which will mediate activated hepatic stellate cells survival (positive SSH α-SMA) and cause hepatic collagen deposition.[6]

Mesenchymal cells initiate an inflammatory response by increasing the number of leukocytes that recruit chemokines and adhesion molecules, perform phagocytosis, antigen presentation, and T cell activation.[3] Liver sinusoidal endothelial cells (LSEC) also play a role in inflammation by acting as a conduit for pro-inflammatory chemicals. in the regeneration and fibrosis of the liver. In response to acute injury, LSEC activates the chemokine receptor CXCR7, which collaborates with CXCR4 signals via the DNA-binding protein inhibitor ID1 to produce pro-regenerative signals such as hepatocyte growth factor (HGF), which promotes hepatocyte enlargement. In chronic damage, constitutive signals from fibroblast growth factor receptor 1 (FGFR1) will reduce the ratio of CXCR7 to CXCR4 expression, take over the

activation of inhibitor of DNA binding 1 (ID1), and trigger SSH proliferation by triggering the secretion of cytokines such as TGF-β, bone morphogenetic protein 2 (BMP2), Platelet-Derived Growth Factor (PDGF).[2]

Macrophages in the liver also play a role in the fibrogenesis and extracellular matrix resolution process. Proinflammatory macrophages will transform into restorative macrophages, increasing the production of matrix metalloproteinases (MMP), including MMP9 and MMP12, as well as genes related to phagocytosis and growth factor.[5] As a result, alterations in macrophage phenotype and the elimination of profibrotic macrophages play an important role in extracellular matrix resorption and liver regeneration during acute inflammation.[2] Changes in macrophage phenotype do not occur in chronic inflammation, yet there is persistent interaction between various inflammatory agents. This allows the process of liver fibrosis to occur concurrently with regeneration.

The Role of Stem Cells for Liver Regeneration

The main treatment for end-stage liver cirrhosis is liver transplantation. Facilities that can perform liver transplants in Indonesia are still very limited and often with long waiting times or queues for transplants as well as very high costs. Therefore, it is often not possible for patients to undergo liver transplantation.[7] On the other side, stem cell transplantation may be a potential strategy to prevent disease progression and improve the degree of fibrosis. Studies show that liver cells can regenerate, although it requires a balance between secreted matrix proteins and matrix metalloproteinase (MMP).[8]

Stem cells can divide and differentiate into various derivatives, thus, can be an option in regenerative medicine, especially in the liver. Various stem cell derivatives that have been studied include pluripotent embryonic stem cells, hematopoietic stem cells, mesenchymal stem cells, and so on.[8] Administration of embryonic stem cells has the potential for malignancy such as splenic teratoma so that its application is limited.[9] Hematopoietic stem cells are only sourced from the hematopoietic system thus its clinical application is also limited.[8]

Liver regeneration due to chronic liver damage is also played by mesenchymal cells through the mesenchymal-epithelial or epithelial-mesenchymal transition (MET/EMT) process.[8,10] The liver consists of mesenchymal and epithelial cells, and successful liver regeneration occurs when damaged liver epithelial cells are replaced by new epithelial cells. Epithelial cells are adherent and have apico-basal polarity, while mesenchymal cells are non-polar and can migrate due to a lack of intercellular connections. During regeneration a MET/EMT process will occur which results in changes in cell properties, especially its plasticity.[11]

There are 3 types of MET/EMT processes. Type 1 occurs during implantation, embryogenesis and organ development where mesodermal and endodermal mesenchyme occurs which will form secondary epithelium that forms the organs. Type 2 is associated with cell damage and inflammation, a process involving fibroblastic cells which, if inflammation persists, will accumulate and cause progressive fibrosis. Type 3 occurs due to genetic or epigenetic changes that occur in cancer cells and promote invasion and spread of tumor cells. In liver regeneration in general, EMT/MET type 2 occurs which can cause fibrosis, while the desired process is EMT/MET type 1. This is the basis for trans-differentiation of mesenchymal stem cells into hepatocytes, so it can be considered as an alternative therapy for liver regeneration.[12]

The interaction between hepatic progenitor cells and mesenchymal cells is important in liver remodelling, thus, mesenchymal stem cells have therapeutic potential in liver damage.[8] Mesenchymal stem cells can differentiate into hepatic cells and help regenerate liver function, which is characterized by apoptosis of hepatic stellate cells, decreased TGF -β1, and α-SMA gene expression.[13] Damaged liver cells will be surrounded by an extracellular matrix into which the mesenchymal stem cells is embedded, and this matrix will trigger the differentiation of the mesenchymal stem cells into hepatocyte cells assisted by various cytokines and growth factors.[7] Apart from differentiation into hepatocyte cells , various trophic factors are also secreted by mesenchymal stem cells, thereby preventing apoptosis of hepatocytes with the help of antiapoptotic factors, such as: hepatocytes growth factor (HGF), insulin-like growth factor (IGF)-1, angiogenetic factors (vascular endothelial growth factor/VEGF), mitogenic factors (epidermal growth factor/EGF , nerve growth factor/NGF), and TGF-α.[8]

The advantages of mesenchymal stem cells as a therapeutic agent in liver fibrosis include the ability to self-repair, implant in the target area (engraftment), immunomodulation, dual differentiation as well as ability to secrete trophic factors and help restore damaged tissue.[14] Various sources of mesenchymal stem cells have been discovered from various studies , such as from bone marrow, adipose cells, umbilical cord, peripheral blood, synovial membrane, cartilage and amniotic fluid.[7,8] Of these various sources, the most common source that has shown therapeutic potential in liver disease is bone marrow mesenchymal stem cells, umbilical cord mesenchymal stem cells and adipose tissue mesenchymal stem cells.

Long-term outcomes in patients with liver cirrhosis using umbilical cord mesenchymal stem cells show satisfactory results, although in terms of short-term effectiveness bone marrow mesenchymal stem cells is preferred.[15-18] Zhang et al. demonstrated that when using umbilical cord mesenchymal stem cells in humans with follow up period for one

year, showed improvement in liver function as indicated by increased albumin levels, decreased bilirubin levels, and reduced ascites, without any significant side effects.[18] Another study showed that in patients who showed an incomplete response to uresodeoxycholic acid in biliary cirrhosis, there was a decrease in alkaline phosphatase and gamma glutamyl transferase (GGT) within 48 weeks.[17]

Routes of Administration of Mesenchymal Stem Cells

The route of administration of mesenchymal stem cells is very important in therapeutic effectiveness. Mesenchymal stem cells administration can be given through a peripheral vein, intrahepatic, or intrasplenic. Although in vivo studies show good migration of mesenchymal stem cells through peripheral veins into chronically damaged liver parenchyma, mesenchymal stem cells engraftment is limited in acute liver damage.[19] Intravenous administration will result in large cell loss in the capillaries, especially in the lung, resulting in cell shorter lifespan.[7,8] Vascular patency is very influential for mesenchymal stem cells levels in target organs. When given together with heparin, the level of mesenchymal stem cells trapped in the lungs will decrease so that the number of cells going to the liver becomes greater.[8] Another risk of administering mesenchymal stem cells by intravenous route is procoagulant activity, which is related to the expression of tissue factor that when comes into contact with blood, will coagulate and result in thrombosis.[20] In research conducted by Coppin et al., intravenous administration of mesenchymal stem cells resulted in thrombogenic events in one in eleven patients, namely thrombus in the portal vein.[21]

Administration by the intrahepatic route is thought to be the ideal route because fewer cells are lost in the circulation and more mesenchymal stem cells differentiates into hepatocytes in the damaged liver area.[7] Administration via the intrasplenic route can be an alternative, because the splenic vein flows into the portal vein, with easier administration techniques.[22] There are not many studies comparing mesenchymal stem cells administration routes simultaneously. Amer et al. conducted a preliminary study by comparing intrahepatic and intrasplenic mesenchymal stem cells injections in 40 patients with chronic liver failure due to hepatitis C, and showed a significant increase in liver function compared to controls.[23] His study showed that the intrahepatic route was more effective than the intrasplenic route, which was marked by reductions in fatigue and MELD scores, although this effect was only seen in the first month and these differences disappeared in the following months.[22,23] This shows that the intrahepatic pathway allows faster engraftment, but does not affect the total engraftment cells. Technically, intrasplenic injection is easier to do, but has more minor side effects, namely fever that subsides with antipyretics.[23]

Therapeutic Mechanisms of Mesenchymal Stem Cells Transplantation in Liver Disease

Hepatocyte-like cells derived from mesenchymal stem cells are considered as a surrogate source for liver regeneration.[14] Mesenchymal stem cells differentiation into liver cells is influenced by several factors such as: hepatocytes growth factor (HGF), fibroblast growth factor (FGF)-2/4, epidermal growth factor (EGF), oncostatin M, leukemia inhibitory factor, dexamethasone, insulin-transferrin-selenium, or nicotinamide.[24] Damaged liver tissue is surrounded by extracellular matrix which is the location for mesenchymal stem cells engraftment and differentiation. Co-culture with liver cells and pellet culture can induce differentiation into hepatocyte like cells.[25,26] Differentiation of mesenchymal stem cells into hepatocytes occurs in less than 1% of the total liver mass.[27] To increase this number, it is necessary to develop better techniques to trigger the differentiation of mesenchymal stem cells into hepatocytes in the management of liver disease.

Chronic liver damage caused by inflammation is marked by infiltration of T cells, B cells and monocytes, thus, immunosuppressive agents can help liver regeneration before and after liver transplantation.[28,29] The immunomodulatory properties of mesenchymal stem cells can help in a similar way, namely by inhibiting T cells by releasing various factors such as nitric oxide, prostaglandin E (PGE)-2, indoleamine 2, 3-deoxygenase, IL-6, IL-10, and human leukocyte antigen G, thus controlling the proliferation and function of various immune cells.[30] The immunosuppressive ability of mesenchymal stem cells is produced by various combinations of cytokines such as interferon-γ, IL-1α, and TNF-α. In addition, mesenchymal stem cells can inhibit B cell activation, reducing immunoglobulin levels.[31] Mesenchymal stem cells co-culture was associated with significant reduction of chemokine receptors (CXCR4, CXCR7, and CXCR5).[32] Mesenchymal stem cells also induce polarization of inflammatory macrophages into alternative macrophages that produce factors such as IL-10 and IL-1Ra that repair liver damage.[33]

Chronic liver damage also causes trans-differentiation of quiescent mesenchymal stem cells into fibrogenic myofibroblasts that produce excessive matrix proteins that result in fibrosis.[7] Proliferation of activated mesenchymal stem cells and collagen deposition can be inhibited by mesenchymal stem cells by direct cell contact. Through an indirect contact mechanism, mesenchymal stem cells produces several factors such as TGF-β3, TNF-α, IL-10, and HGF which inhibit collagen synthesis, and HGF and NGF promote mesenchymal stem cells apoptosis.[34-36] Mesenchymal stem cells co-cultured with mesenchymal stem cells will inhibit mesenchymal stem cells proliferation and α-SMA expression through cell contact. Mesenchymal stem cells also increase the expression of MMPs that degrade the extracellular matrix, and reduce the expression of TIMPs that inhibit the process.[35]

Risks of Mesenchymal Stem Cells Transplantation

Several studies regarding the administration of mesenchymal stem cells for chronic liver disease have been conducted, and there are special considerations regarding the risks, including the risk of carcinogenesis and viral transmission.[7] Mesenchymal stem cells can secrete various trophic factors and growth factors that trigger the growth of tumor cells such as carcinoma-associated fibroblasts (CAF).[37] Previous animal studies suggest that the risk of malignancy is related to the number of breeding cycles (passages).[37] Although malignant transformation in humans has not been reported, most follow-up periods are still too short for tumors or malignant transformation to occur. For this reason, it is necessary to analyze the integrity of the chromosome before mesenchymal stem cells transplantation.[38]

In the case of allotransplantation, there is a risk of transmitting the virus to the patient. Although transmission of parvovirus B19 to mesenchymal stem cells has been studied in vitro, B19-induced viremia resulting from mesenchymal stem cells transplantation in humans has not been reported. There is no information regarding the transmission of herpes simplex virus (HSV) and cytomegalovirus (CMV) via mesenchymal stem cells in vivo. For this reason, it is necessary to screen for parvovirus B19, HSV, and CMV in both donors and allotransplant recipients because of the possibility of infection, especially in patients who are immunodeficient.[7]

Liver Fibrosis and Regeneration Parameters

Various tests to evaluate liver fibrosis and regeneration have been developed. Aminotransferases, such as alanine transaminase (ALT) and aspartate transaminase (AST), are hepatocellular damage indicators. These two enzymes contribute to gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid, which results in the formation of oxaloacetic and pyruvic acids. As the AST enzyme is found in the cytosol and mitochondrial isoenzymes of the liver, heart and skeletal muscle cells, kidneys, brain, pancreas, lungs, and blood cells, the increase in this enzyme is not specific nor sensitive to the liver. Due to its high concentration in the liver, the ALT enzymes are more specific.[39]

Cholestasis parameters include elevated bilirubin levels, particularly direct bilirubin, alkaline phosphatase, and GGT, which are not proportional to AST and ALT levels. Bilirubin is a derivative of hemoglobin that is an end product of heme catabolism. Indirect (unconjugated) bilirubin is transported to the liver by binding to albumin, where it is conjugated to form bilirubin glucuronide (direct bilirubin). Direct bilirubin is then excreted in the urine after being released into the bile. Alkaline phosphatase is a zinc metalloenzyme found in the microvilli of the biliary canaliculus as well as other tissues such as bone, gut, and the placenta. These parameters might rise as a result of hepatobiliary disorders including bile duct obstruction, as well as non-hepatic causes

like bone disorders, pregnancy, kidney disorders, and malignancy. Since it is not found in bones, Gamma-Glutamyl Transferase (GGT) is an enzyme found in cell membranes that catalyzes the transfer of the gamma-glutamyl group from peptides to other amino acids. It is more specific in detecting biliary diseases than ALP.[39]

Albumin is a protein that is produced in the liver. When there is a disruption, albumin production is diminished. Low albumin levels with normal liver function occur in conditions such as insufficient protein intake in malnutrition or excessive protein loss in nephrotic syndrome, malabsorption, or enteropathy.[39] Prothrombin time (PT) measures the rate at which prothrombin is converted to thrombin and represents the liver synthesis. In addition to factor VIII, coagulation factors are produced in the liver, therefore prolonged PT may occur in hepatic diseases, indicating the activity of factors II, V, VII, and X.[39]

A histopathological examination could determine the extent of liver fibrosis. Fibrosis plays a role in chronic inflammation in the liver. Persistent inflammation leads to the deposition of connective tissue in the parenchyma, which replaces the normal liver architecture.[3] Histopathological examination with hematoxyllin-eosin staining or histochemical staining (Masson's trichrome or Sirius Red) demonstrates the liver fibrosis with varying degrees of deformation of liver architecture.[40] Various scoring systems measuring the degree of fibrosis have been established over time, including the Scheuer system, Batts- Ludwig, Ishak, and METAVIR.[40] The Laennec system is a modified version of the METAVIR system that categorizes stage 4 into 4A, 4B, and 4C based on the thickness of the septa and the size of the nodules on the liver biopsy.[40] The Laennec criteria can be seen in **Table 1** below. [41]

Table 1. Laennec Scoring System for Liver Biopsy Fibrosis[41]

Stage	Classification	Septa (thickness and amount)	Criteria	Score
0	No definite fibrosis			0
1	Minimal fibrosis	+/-	Absent septa or thin septa are uncommon, portal expansions or mild sinusoidal fibrosis may be present.	1
2	Mild fibrosis	+	Some septa are thin; portal expansion or mild sinusoidal fibrosis may occur	2
3	Moderate fibrosis	++	Moderately thin septa; to	3

			partial cirrhosis	
4A	Cirrhosis, mild, definite or probable	+++	Septation is significant with a round contour or visible nodule. The majority of septa are thin	4
4B	Moderate cirrhosis	++++	At least two broad septa, although not very extensive, and tiny nodules occupy less than half of the length of the sample	5
4C	Severe cirrhosis	+++++	At least one very large septum and more than half of the length of the sample comprised of tiny nodules (micronodular cirrhosis)	6

Fibrosis regression may occur, which is characterized as a decrease in the fibrosis score on consecutive biopsies using any scoring system. This regression can be used as a benchmark for final outcomes in numerous therapeutic clinical trials, however, it has limitations in terms of sample size and number of portal tracts (minimum 2 cm or longer, with a minimum of 11 complete portal tracts).[40]

At the cellular level, liver regeneration consists of compensatory hypertrophy followed by hepatocyte hyperplasia. The regeneration process is divided into three stages: initiation (0-5 hours after injury), proliferation (up to day 6), and termination.[42] This injury will initiate a signal cascade that mobilizes immune cells to remove dead tissue, alter metabolic processes, and promote regeneration through the action of numerous cytokines and growth factors.[43]

Initial hemodynamic alterations result from changes in portal venous flow quantity and quality, which activate the regeneration cascade. Increase in portal volume causes shear stress and reduces arterial blood flow. The concentration of lipopolysaccharide (LPS) produced from intestinal bacteria in the portal circulation increases with innate immune activation, promoting growth factors such as HGF and EGF, as well as cytokines such as IL-6 and TNF. Furthermore, intrahepatic volume and shear stress increase urokinase plasminogen activator (uPA), activate the extracellular matrix that binds to HGF, and activate HGF and EGF receptors. Quiescent hepatocytes will then begin the cell cycle, progressing from G0 to G1. Hepatocytes will then produce VEGF, FGF-1 and -2, and angiopoietin-1 and -2 to stimulate endothelial cells; PDGF to activate SSH; and TGF-α to stimulate biliary epithelial cells.[43]

In the proliferation phase, hepatocytes and cholangiocytes proliferate within 72 hours. Angiogenesis begins within 2-3 days as a result of SSH, EC, and Kupffer cells growing in response to hepatocyte cytokines and growth factors. In order to preserve appropriate liver mass and function, antiproliferative substances such as TGF-β produced by SSH and Kupffer cells prevent autonomous proliferation of hepatocytes during the termination phase.[43]

Various cytokines, growth factors, and biological markers derived from the various complex interactions in regeneration may indicate the liver regeneration. Hepatocyte growth factor is a mitogen produced by mesenchymal cells that promotes liver cell proliferation and angiogenesis, therefore its levels in blood rise during acute injury and return to normal after seven days.[43] Tumor necrosis factor- and interleukin-6 (IL-6) are proinflammatory cytokines that aid in liver regeneration.[44] Heparin-binding EGF-like growth factor is a form of EGF produced by endothelial cells and Kupffer cells that aids in liver regeneration, particularly on the fifth to seventh day following liver injury.[43] Vascular endothelial growth factor promotes angiogenesis and neovascularization during liver regeneration and rises in the advanced phase.[45]

Insulin-like growth factor has mitogenic properties that play a role in liver regeneration, especially in chronic liver damage.[46] Fibroblast growth factor is important in biliary homeostasis.[43] Angiopoetin has a role in angiogenesis through Tie-signal 1 and Tie-2 receptor tyrosine kinases.[47] Platelet-Derived Growth Factor promotes cell growth, however blocking its receptor does not totally disrupt liver regeneration, therefore its role appears to be taken over by other growth factors[48].

Proliferating cell nuclear antigen (PCNA) and Ki-67 are biochemical indicators that are able to predict liver regeneration. Proliferating cell nuclear antigen is a non-histone protein found in the nucleus that aids in DNA synthesis and cell cycle progression. Studies on animals found an accumulation of PCNA-positive cells in post-hepatectomy animals, indicating liver growth or regeneration.[49] Ki-67 is a cell cycle core protein whose expression signifies cell division. [43]

Studies show that several proteins such as the specific protein on the bile duct cytokeratin 19 (CK19) and the hepatocyte specific protein HepPar1 are progenitor cells in liver morphogenesis and thus active in liver regeneration.[50] These proteins may serve as indicators of liver regeneration.

Conflict of Interest

None declared.

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