

Clinical and Preclinical Research Using Autologous Cell Therapy for Chronic Liver Disease

BACKGROUND

Chronic liver disease is an increasing problem in Japan. There are currently an estimated 2 million persons carrying Hepatitis C virus (HCV) and 1.5 million carriers of Hepatitis B Virus (HBV). It has also been estimated that 20-30% of the population have non-alcoholic fatty liver disease¹ and are at risk of developing Non-Alcoholic Steatohepatitis (NASH), which is accompanied by chronic hepatic inflammation with stellate cell activation and succeeding hepatic fibrosis. NASH is frequently associated with Metabolic Syndrome which is the most important and urgent national health problem in Japan today. Indeed, it is estimated that one million people are currently affected by NASH.

Unlike many other organs, for example the stomach and intestines, regeneration of liver cell mass following an injury occurs primarily through replication of mature cells rather than from a pool of stem and progenitor cells. This replicative capacity is easily capable of restoring liver mass after partial hepatectomy in a matter of days. However, repetitive injury such as that occurring in chronic viral hepatitis or injury where the intrinsic proliferative capacity of hepatocytes is impaired or constrained can overwhelm the liver's endogenous regenerative ability and lead to cirrhosis and liver failure. At present these conditions can be addressed satisfactorily only by liver transplantation. While living donor liver transplants have

improved availability of tissue for transplant² alternative strategies to restore hepatocyte number, augment liver function, and counteract progressive hepatic fibrosis are still urgently needed.

Although approaches such as a bioartificial liver, xenogeneic transplant, and embryonic stem cells have the potential to help these patients, autologous cell therapy is a far preferable strategy. One key barrier to this approach is the identification of a clinically acceptable source of autologous cells with the capacity to positively impact liver disease. In malignant disease, where the uninvolved portions of the organ remain essentially healthy, the liver itself can be the source of replacement cells so that interventions such as transhepatic percutaneous portal vein embolization can be used to promote hepatic regeneration in advance of a planned major resection³. However, this process is of limited utility in chronic liver disease where prolonged activation of the hepatic wound healing process leads to generation of extensive fibrosis and impairment of the endogenous regenerative capacity of the liver.

CELL THERAPY IN EXPERIMENTAL MODELS OF HEPATIC FIBROSIS

A number of studies have described use of cell therapy in animal models of hepatic fibrosis. Thus, several groups have demonstrated improvements in liver function, serum transaminase levels, and hepatic fibrosis

in animal models of liver injury that were treated with bone marrow mononuclear cells, Endothelial Progenitor Cells (EPCs), Mesenchymal Stem Cells (MSCs; also referred to as Marrow Stromal cells), or Adipose-Derived Stem Cells (ADSCs)⁴⁻¹⁴.

For example, Banas et al have shown that ADSCs (a cultured cell population from adipose tissue that has properties similar, but not identical, to MSCs) can be induced to differentiate into cells with many of the key functional properties of hepatocytes including expression of albumin and clearance of ammonia¹². Intravenous delivery of these cells resulted in improved liver function in a rat CCl₄ injury model^{12,13}. Similar findings have been reported by a second group which also showed that delivery through the portal vein was more effective than that via the peripheral venous system¹⁴. Interestingly, in the studies by Banas et al, ADSCs that had not been differentiated along the hepatic lineage exhibited similar ability to improve function. This observation, combined with the very low number of donor cells retained within the liver suggests that the primary mechanism of action is not a direct replacement of parenchymal hepatocytes, but an indirect role such as induction of stellate cell quiescence or preservation of hepatocytes. In contrast to this observation, another group has shown that pre-differentiation of human ADSCs towards a hepatocytic phenotype results in improved engraftment into the livers of immunodeficient mice. However, this study

Table 1: Summary of Clinical Trials of Cell Therapy for Liver Disease

Disease State (number of patients)	Cell Type	Delivery Route (Cell Dose)	Outcome	Ref
Liver cirrhosis (9)	Bone marrow mononuclear cells from 400mL marrow	Peripheral vein (5.2±0.6x10 ⁹)	Improved serum albumin, total protein, and Child-Pugh score at 24wks.	16,17
Chronic liver disease (10)	Bone marrow from ~50mL marrow	Hepatic artery (1x10 ⁸)	Modest improvement in bilirubin (2.8±1.2 to 2.1±0.9) and serum albumin (3.5±0.5 to 3.7±0.5) at 4 months	18
Liver insufficiency (4)	CD34-selected mobilized peripheral blood cells	Hepatic artery (1x10 ⁷)	Improved albumin, bilirubin, and ALT at one month follow-up	19
Alcoholic liver cirrhosis (9)	Cell culture-expanded CD34-selected mobilized peripheral blood cells	Hepatic artery (2.3x10 ⁸)	Decreased bilirubin at 4, 8, and 12 weeks. Improved transaminases. 7 of 9 had improvement in Child-Pugh score and 5 of 9 had improvement in ascites.	20
Liver insufficiency (5 total; 4 alcoholic cirrhosis and 1 cryptogenic)	CD34-selected mobilized peripheral blood cells	Hepatic artery or portal vein (106-2x10 ⁸)	4 patients showed initial decrease in bilirubin at 6 months. Bilirubin increased in 3 of these at 12 months and in the 4th by 18 months.	21
Decompensated cirrhosis (4)	CD34-selected bone marrow cells from ~200mL marrow	Hepatic artery 3-10x10 ⁶	2 patients showed mixed improvement, one patient worsened, and 1 died of radiocontrast nephropathy and subsequent hepatorenal syndrome	22
Mixed Liver Cirrhosis (total 8; 4 HBV, 1 HCV, 1 alcoholic cirrhosis, and 2 cryptogenic)	Autologous cultured MSC	Peripheral or portal vein (3-5x10 ⁷)	Improved Model End-Stage Liver Disease score (from 17.9±5.6 to 10.7±6.3). Improved serum creatinine.	23
Liver cirrhosis (total 4; three cryptogenic, 1 autoimmune hepatitis)	Autologous cultured MSC	Peripheral vein (3.2x10 ⁷)	2 patients showed modest improvement in Model End-Stage Liver Disease score (from 16 to 12 and from 17-14 as measured at 12 months). All 4 showed improvement in QoL as assessed by SF-36	24

did not evaluate liver function or examine engraftment in the context of liver injury¹⁵. The observation that undifferentiated ADSCs improve liver function is consistent with a report that showed that undifferentiated MSCs exhibited greater anti-fibrotic effect than differentiated MSCs in CCl₄-induced liver injury in the rat¹⁰.

CLINICAL RESEARCH USING CELL THERAPY FOR LIVER DISEASE

A small number of clinical studies has been initiated using a range of cell types for advanced liver disease¹⁶⁻²⁴ (Table 1). The results show indications of efficacy although the stability of the effect is not clear, as only a few studies have looked beyond 4-6 months. In two longer term studies there appears to be a trend towards worsening of disease at or beyond 12 months after treatment^{21,24}.

Overall the data are promising but suggest that optimization of design features such as the route of administration and the use of repeated treatments may be necessary. This is consistent with animal data showing that route of administration directly impacts efficacy^{14,25} and that repeated doses may increase efficacy⁶. It should also be noted that all of the clinical studies performed to date are limited by the absence of conventional randomization and control groups. There are currently no reports of clinical studies using cells isolated from adipose tissue. However, a group in Kanazawa, Japan, under the direction of Dr Yoshio Sakai has initiated recruitment in a study to evaluate the use of freshly-isolated (non-cultured) adipose derived stem and regenerative cells (ADRC) for patients with liver cirrhosis (Study Identifier NCT00913289 at <http://clinicaltrials.gov>).

POTENTIAL MECHANISM OF ACTION OF CELL THERAPY IN HEPATIC FIBROSIS

Hepatic fibrosis arises through an inflammatory process in which incoming activated leukocytes induce hepatic stellate cells to convert into pro-fibrotic myofibroblasts (reviewed by Friedman²⁶). Co-culture studies have shown that MSCs are capable of modulating stellate cell proliferation and collagen synthesis²⁷. Further, administration of ADSCs has been shown to reduce inflammation in a number of experimental settings including colitis²⁸, rheumatoid arthritis^{29,30}, allergic rhinitis³¹, hemorrhagic stroke³², and myocardial ischemia³³. Clinical studies with ADSCs have demonstrated the ability to induce healing of fistulas in the context of inflammatory bowel disease and Crohn's Disease^{34,35}. ADSCs can

also modulate hepatic fibrosis through the expression of enzymes and inhibitors involved in remodeling of the ECM or by altering the function of activated hepatic stellate cells and myofibroblasts. Collagenases such as MMP-1, MMP-8, MMP-13, and MMP-14 (all of which have been shown to be expressed by ADSCs³⁶) are particularly important as they possess the ability to degrade fibrillar collagen and thereby counteract progression of fibrosis.

Four of the clinical studies cited in Table 1 used marrow and blood cells isolated on the basis of expression of the cell surface molecule CD34¹⁹⁻²². This population includes hematopoietic stem and progenitor cells³⁷ and endothelial progenitor cells (EPCs)³⁸. As mentioned above, EPCs have been shown to improve survival and hepatic function in animal models of liver disease^{5,6,39}. It is likely that the EPCs act, at least in part, by promoting new vessel formation and improving hepatocyte perfusion. Liver cirrhosis involves establishment of intrahepatic vascular shunts that can dramatically reduce hepatocyte perfusion and create a hypoxic environment that is hostile to regeneration and repair. Indeed, the development of extensive intrahepatic shunts has been described as the major determinant of the point of no return for cirrhosis⁴⁰. EPCs likely act by improving the microenvironment within the liver through formation of new vessels and extracellular matrix remodeling. This creates a microenvironment in which normal liver repair mechanisms are able to operate more effectively as evidenced by increased hepatocyte proliferation in EPC-treated animals^{6,39}.

FRESHLY-ISOLATED ADIPOSE-DERIVED REGENERATIVE CELLS (ADRC)

ADSCs are generated by digesting adipose tissue, removing the adipocytes, and placing the remaining cells in culture over a period of weeks to create an adherent cell population

with many similarities to MSCs. However, analysis of the cell population prior to cell culture has shown that it contains a very high stem cells frequency (~1-2% of nucleated cells⁴¹⁻⁴⁵). This in contrast to age-matched bone marrow where stem cells comprise only 0.0004% of nucleated cells^{46,47}. The freshly isolated population also contains EPCs⁴⁸. This fact, combined with ability to obtain relatively large volumes of tissue with minimal morbidity through liposuction, suggests that this population (referred to as Adipose-Derived stem and Regenerative Cells; ADRCs) is a clinically relevant dose of stem and regenerative cells for autologous cell therapy. Indeed, ADRCs have been examined in a number of clinical settings including the repair of a tracheomediastinal fistula⁴⁹, repair of a calvarial defect⁵⁰, multiple sclerosis⁵¹, facial rejuvenation⁵², and breast reconstruction⁵³. ADRCs have also demonstrated utility in preclinical models of myocardial ischemia^{54,55}. More recently, it has been shown that intra-arterial administration of ADRCs significantly improves renal function and survival in a rat model of acute renal ischemia⁵⁶. Thus, whereas survival of control animals at day 7 was only 56%, survival of animals treated with ADRC was 100%. These findings suggest that freshly-isolated cells have similar potential to modulate inflammation and may be of utility in modulating hepatic fibrosis. Clinical trials of ADRCs have also been initiated in Liver Cirrhosis (Clinicaltrials.gov Study Identifier NCT00913289), Acute Myocardial Ischemia with ST segment elevation (Study Identifier NCT00442806), Non-Revascularizable Myocardial Ischemia (Study Identifier NCT00426868), and Breast Reconstruction following partial mastectomy (Study Identifier NCT00616135).

PRACTICAL ISSUES ASSOCIATED WITH CLINICAL USE OF FRESHLY ISOLATED CELLS

While the data described above demonstrate

a clear potential for ADRC in liver disease there are factors that need to be considered before initiation of clinical studies. First, as with almost all preclinical models, the results described above were obtained using otherwise healthy animals. This is a distinctly different setting than that in the clinic where the progressive nature of the underlying disease may negatively impact the ability of the cells to act or the liver to respond. It should be noted, however, that this potential limitation does not appear to have prevented efficacy in the early clinical studies described above and there is no reason to believe that this would be different for cells obtained from adipose tissue.

Second, adipose tissue is a solid organ rather than a suspension of single cells like marrow or blood. Consequently, cells from adipose tissue must be obtained by enzymatic digestion. This requires application of clinical grade enzymes and reagents and use of a tissue processing method that minimizes the risk of contamination of the cells during processing. Further, for intra-vascular delivery the systems and methods used to generate and deliver these cells should be validated to ensure that delivery can be achieved without risk of embolism or other adverse event.

SUMMARY

In summary, cells obtained from human adipose tissue have been shown to improve hepatic function in animal models of liver disease. Data from other models suggest that cell culture may not be necessary in order to obtain a clinically effective population and may even be deleterious by eliminating other populations that may contribute to a positive outcome. Clinical use of freshly isolated cells requires application of tissue collection, processing, and cell delivery systems and methods that minimize the potential for harm to the recipient.

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