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Review Article

Hepatic Stellate Cells Targeting- A Specific Approach to Liver Cirrhosis

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ABSTRACT

Cirrhosis is one of the chronic generalised disease and has a variety of clinical manifestations and complications some of which can be a life threatening. This results in decrease in hepatocellular mass and thus functions. It is 12th leading cause of death in United States. Hepatic stellate cells (HSC) plays a crucial role in the development of liver fibrosis because of their prominent role in extracellular matrix production ,regulation of vascular tone, and production of inflammatory mediators such as transforming growth factor-b (TGF-b) and platelet-derived growth factor (PDGF).Therefore, these cells are major target for the treatment of Cirrhosis.

Anti-fibrotic drugs are not efficiently taken up by HSC or may produce unwanted side-effects outside the liver. Cell-specific delivery can provide a solution to these problems, but a specific drug carrier for HSC has not been described until now. The mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor, which is expressed in particular upon HSC during fibrosis, may serve as a target-receptor for a potential carrier.

The primary aim of drug targeting is to manipulate the whole body distribution of drugs, that is, to prevent distribution to non-target cells and concomitantly increase the drug concentration at the targeted site. Carrier molecules are designed for their selective cellular uptake, taking advantage of specific receptors or binding sites present on the surface membrane of the target cell.

INTRODUCTION

Liver Cirrhosis is the end stage of all the diseases like viral hepatitis, alcohol abuse, non-alcoholic steatohepatitis and other diseases [1]. In Liver Cirrhosis acute liver damage leads to chronic inflammation and fibrosis. Unfortunately there is no treatment currently available for liver fibrosis apart from organ transplantation but in that also donor organ shortage and high costs remain a serious problem. Hepatic failure after transplantation is still burdened by a high mortality rate. So finding a proper pharmacotherapeutic treatment for the liver fibrosis is very challenging.

Selective targeting of anti-fibrotic drugs to hepatic stellate cells (HSC) has recently been proposed which are identified as the key fibrogenic cell type in the progression of Cirrhosis [2-4].

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With the help of targeted drug delivery system potent antifibrotic drug can be delivered intracellular within the diseased liver and even within the desired cell type. For this mannose-6-phosphate modified albumin (M6PHSA) has been proposed which binds with high affinity to the insulin-like growth factor II/mannose-6-phosphate receptor on activated HSC [5].

The current status of Cirrhosis disease

- Cirrhosis is one of the major disease affecting millions of people world-wide.
- Cirrhosis is a chronic liver disorder caused by a variety of diseases, with the most common being hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, and alcoholic liver disease. These disease attack the liver, leading to progressive liver damage and, ultimately, liver failure and death. For example, 1–46% of patients with chronic HCV infection will likely develop cirrhosis during a 30-year period.
- Cirrhosis, the twelfth leading cause of death in the United States in 2007, represents a large economic burden, with the national

cost for treatment in 2008 ranging from $14 million to $2 billion, depending on disease etiology. This has been estimated that the burden is expected to rise over the next 20 years, given that the percentage of patients with HCV-related cirrhosis is predicted to almost double.

The overall cost of cirrhosis includes direct costs (drug and hospitalization costs) and indirect costs (due to loss of work productivity and reduction in health-related quality of life [HRQOL]). In 2004, the direct costs of cirrhosis and chronic liver disease in the United States (excluding patients with HCV infection) were estimated to be $2.5 billion, whereas indirect costs were estimated to be $10.6 billion. Because cirrhosis is a progressive disorder, preventing or arresting its causes may substantially reduce the monetary burden of the disease. Given that liver transplantation entails a large economic outlay for relatively few individuals, the cost-effectiveness of the procedure, particularly in terms of the allocation of available livers and patient's HRQOL post-transplantation, may be questionable [6].

Functional Classification of Cirrhosis

On the basis of their anatomy and pathogenesis Cirrhosis can be divided into following categories:

- Alcohol Cirrhosis.
- Cirrhosis following viral hepatitis.
- Chemically (drug) induced Cirrhosis.
- Obstructive biliary Cirrhosis.
- Congestive Cirrhosis.
- Neural hepatic Cirrhosis.
- Cirrhosis from iron over load (Hemochromatosis).
- Cirrhosis on hereditary basis.
- Multi factorial Cirrhosis.
- Cirrhosis on unknown etiology.

Hepatic Stellate Cells – Ultrastructure and Retinoid Storage

- Hepatic stellate cells are located in the sub endothelial space, between the basolateral surface of hepatocytes and the anti-luminal side of sinusoidal endothelial cells. They comprise approximately one-third of the non parenchymal cell population and 15% of the total number of resident cells in normal liver.
- Stellate cells in normal liver have spindle-shaped cell bodies with oval or elongated nuclei.
- Ultra structurally, there have moderately developed rough endoplasmic reticulum (rER), juxtanuclear small Golgi complex, and prominent dendritic cytoplasmic processes. The sub endothelial processes wrap around sinusoids between endothelial cells and hepatocytes. On each of these processes, there are numerous thorny micro projections (spines). The function of these projections had been obscure until a recent, elegant study has demonstrated that these protrusions serve a vital role as the cell's leading edge in "sensing" chemotactic signals, and then transmitting them to the cell's mechanical apparatus to generate a contractile force.

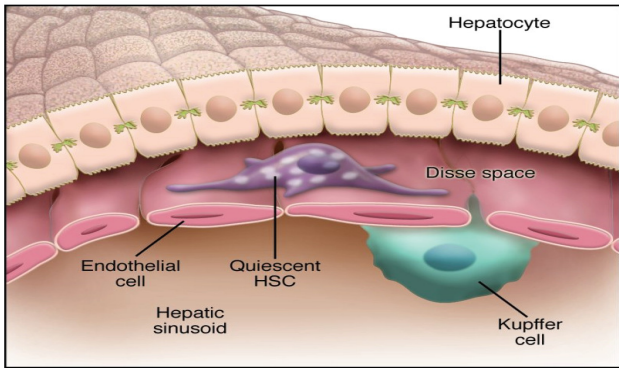

Figure1: Hepatic stellate cells

- A single stellate cell usually surrounds more than two nearby sinusoids. On the other side of the cell (i.e. the anti-luminal surface), multiple processes extend across the space of Disse to make contact with hepatocytes. This intimate contact between stellate cells and their neighbouring cell types may facilitate intercellular transport of soluble mediators and cytokines.

Retinoid Storage

- The most characteristic feature of stellate cells in normal liver is their cytoplasmic storage of vitamin (retinoid) droplets.
- The number of droplets varies with the species and the abundance of vitamin A stores of the organism.
- During liver injury, the fine structure of stellate cells changes considerably. They lose their characteristic droplets and become "activated".
- The activated stellate cells then evolve into myofibroblast-like cells with newly formed collagen fibrils surrounding them.

Fibrogenic Cells of the Liver

- Activated hepatic stellate cells show de novo fibrogenic properties, including proliferation and accumulation in areas of parenchymal cell necrosis, secretion of proinflammatory cytokines and chemokines, and synthesis of a large panel of matrix proteins and of inhibitors of matrix degradation, leading to progressive scar formation (Fig. 2).
- Hepatic myofibroblasts are another source of fibrogenic cells that derive from fibroblasts of the portal connective tissue, perivascular fibroblasts of portal and central veins, and periductular fibroblasts in close contact with bile duct epithelial cells.

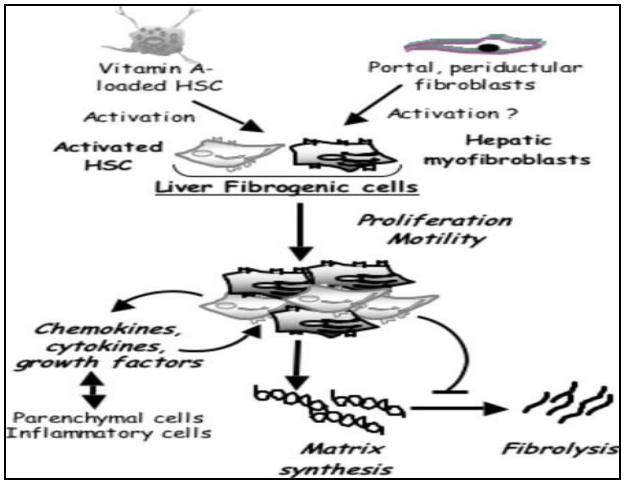

Figure 2: Main properties of Fibrogenic cells of Liver [30]

- Cell-specific expression of these markers has also been described in experimental models and suggests that hepatic myofibroblasts derived from portal (myo) fibroblasts are present within fibrotic septa, whereas activated hepatic stellate cells are found in the subendothelial sinusoidal space close to portal tracts.
- Further work is needed to fully delineate the precise contribution of each cell type to the fibrogenic process, and characterization of the fibrogenic cell lineage may provide useful information. In this respect, recent studies indicate that as yet undefined bone marrow cells constitute a significant source of hepatic stellate cells.
- In addition, bone marrow myofibroblasts represent a significant proportion of hepatic myofibroblasts incirrhosis of diverse etiologies [30].

FUNCTIONS OF HEPATIC STELLATE CELLS IN NORMAL LIVER

- Role in liver development and regeneration
- Retinoid metabolism
- Immunoregulation
- Secretion of lipoproteins, growth factors, and cytokines
- Biology of membrane and nuclear receptors
- Adipogenic features
- Detoxifying and antioxidant enzymes, pH regulation, and generation of oxidant stress
- Transcriptome and proteome analyses [7].

M6PHSA as a soluble carrier protein

- Albumin is the most abundant plasma protein, and has a biological half-life of 19 days.
- It consists of a single chain of 585 amino acids organized in a tri dimensional structure in a helical conformation. The helices are bound by 17 di-sulphide bridges, leaving only one free thiol (Cys34) [8].
- Albumin is biodegradable and therefore biocompatible and contains many different functional groups, i.e. -NH_2 of the lysine residues or methionine, which can be used for conjugation of the homing device, the linker, or the drug.
- In addition, due to its size and charge, it is not cleared from the blood by renal filtration.
- In our strategy, albumin was modified with sugar mannose-6-phosphate groups on its surface resulting in M6PHSA. M6PHSA has been shown to specifically interact with mannose 6-phosphate/insulin-like growth factor II (M6P/IGFII) receptors expressed on the surface of hepatic stellate cells.
- Due to stellate cell proliferation during liver fibrosis and a concomitant increase in M6P/IGF II receptor expression on this cell type [9], the disease process itself may selectively direct the carriers to the diseased tissue.
- This targeting strategy may largely contribute to the increased therapeutic concentration of drug in the target tissue.

HSC targeting materials

- In HSC-selective targeting strategies, the receptor expression for some growth factors on the cell surface is drastically upregulated.
- Examples of receptors that are upregulated on activated cells are the platelet-derived growth factor (PDGF) receptors, the mannose-6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor and many

receptors that allow HSCs to interact with the surrounding ECM. Thus, HSCs form an attractive cellular target for the treatment of hepatic fibro genesis.

- In several recent studies, some modified albumins have been used to target drugs to HSCs: losartan, mycophenolic acid, DOX, 15d-prostaglandin J2, gliotoxin, the viral vector HVJ, pentoxifylline, IL-10 and a kinase inhibitor.
- Most of these constructs displayed antifibrotic effects *in vivo*.
- Losartan-M6PHSA was synthesized by a method in which M6PHSA was prepared as follows: HSA was modified with mannose-6-phosphate groups. Briefly, p-nitro phenyl-a-Dmannopyranoside was phosphorylated and after reduction of the nitro group it was coupled with HAS [10].
- In other method prepared M6P-modified albumin, which was purified using an Amicon Stirred Cell (Amicon, Danvers, MA, USA) followed by Sephadex G-25 gel chromatography (Pharmacia, Uppsala, Sweden)[11].
- The results demonstrated that animals receiving losartan-M6PHSA showed losartan levels that corresponded to 81% of the last injected dose, which was at least 20% of the cumulative dose, while oral losartan yielded liver tissue levels corresponding to only 4% of the cumulative dose (15% of the last dose administered). These results illustrate the preferential hepatic accumulation of losartan-M6PHSA [13].

Isolation of Hepatic stellate cells

- Here firstly the livers of male Wistar rats (450–550 g) were perfused with Gey's balanced salt solution (GBSS) containing collagenase P (Roche Molecular Bio chemicals, Mannheim, Germany), Pronase (Merck, Darmstadt, Germany), and DNase (Roche Molecular Bio chemicals).
- The HSC were separated from the other hepatic cells by density gradient centrifugation and collected at the top of an 11% Nycodenz solution (Nyegaard, Oslo, Norway).
- The cells were then cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc., Paisley, Scotland) containing 10% foetal calf serum, 100 units/ml penicillin, and 100 mg/ml streptomycin.
- After 2 days, cell debris and nonadherent cells were removed by washing and the medium was changed every 2 or 3 days thereafter.
- Cells cultured for 2 days after isolation represented quiescent HSC, whereas those cultured for 10 days after isolation represented activated HSC [13].

Drug targeting to hepatic stellate cells (HSc)

- The use of cell-specific drug carriers to HSC, whose contractile characteristics significantly contribute to the increased intrahepatic resistance and hence portal pressure, may open new therapeutic options in this area.

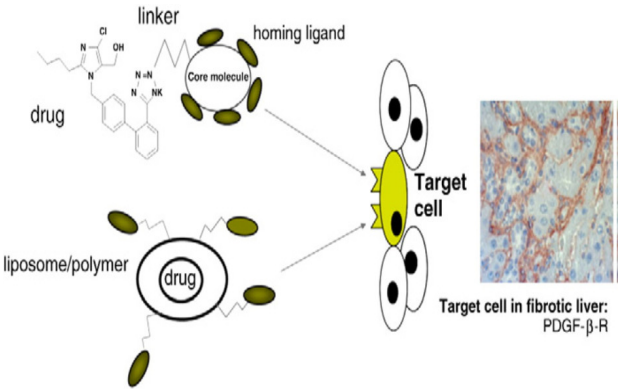

Figure 3: Drug targeting technique to target cells using carriers

- However, this cell type is not easily accessible for drugs, and for this reason many drugs that showed antifibrotic activities *in vitro* were ineffective *in vivo*.
- To optimize the therapeutic success of potential antifibrotic drugs, targeting to hepatic stellate cells has been explored [14].
- Binding sites expressed on (activated) HSC were considered for their ability to serve as potential targets for carrier molecules.One of these is the mannose 6-phosphate/insulin like growth factor II (M6P/IGF-II) receptor, whose expression is increased on activated rat HSC, particularly during fibrosis.
- About 10-20% of the receptor is expressed at the cell surface, and the receptor has binding sites for IGF-II and M6P-containing ligands such as latent transforming growth factor b (L-TGFb), proliferin, and lysosomal enzymes.
- This cation-independent type of M6P receptor plays a role in the lysosomal enzyme targeting and the regulation of cell growth.In addition, a soluble form of the M6P/IGF-II receptor is found in rat and human serum. The main source of this

soluble receptor in adult rats appears to be activated HSC [15].

Modified albumin-based carriers

- The first carrier that accumulated in HSC was reported in 1999 which was recognized as a fundamental new approach and since then several other drug carriers to this cell type have been developed. Cell-specificity for activated HSC in fibrotic livers was obtained by using albumin-based carriers that bind to receptors which are highly up regulated on activated HSC:
  - mannose-6-phosphate/insulin-like growth factor II receptor
  - collagen type VI receptor
  - Platelet derived growth factor β (PDGF-β) receptor.

To reach these receptors, albumin molecules were substituted with mannose-6-phosphate (M6P) or with peptides that recognized either the PDGF-receptor or the collagen type VI receptor.

Mannose6-phosphate residues have also been applied for the delivery of siRNA to HSC, although this has only been demonstrated *in vitro. In vivo* delivery of siRNA to HSC has been demonstrated in a study of using liposomes substituted with vitamin A.

- In normal livers, quiescent HSC store vitamin A and these authors used vitamin A as a homing device to deliver liposomes to activated HSC in fibrotic tissue. This is the first study showing effective delivery of siRNA *in vivo* and it may represent an important step in siRNA-based therapies although further confirmation of this study is still awaited [17].

Specific Delivery of Therapeutic Cytokines to HSC

- The targeting to HSC via the M6P homing device was also used to deliver a cytokine to HSC.Interleukin-10, a cytokine endowed with anti-inflammatory and anti-fibrotic activities was successfully delivered to HSC by coupling M6P-residues to it.
- This re-direction of cytokines opens many new opportunities for the clinical application of these powerful mediators. The most recent and important illustration is the use of Interferon γ (IFNγ) for antifibrotic purposes. IFNγ is a cytokine with potent antifibrotic activities but its clinical use is limited due to its many adverse effects induced by the uptake of this cytokine in nearly all cells of the body.
- Efficacy is also limited by the short half-life of IFNγ which could not be overcome by dose increments because of the adverse effects. By coupling of PDGF-β receptor binding peptides to this cytokine, accumulation of IFNγ in HSC was achieved and its antifibrotic effects were strongly enhanced while adverse effects were completely abolished.

Other Approaches to Target Hsc

- In this approachM6P is combined with a ***RGD homing device***. This RGD sequence is also present in the collagen type VI receptor recognizing peptide. Studies with HSC in cultures proved that M6PRGD constructs were more effective in inhibiting fibrotic activities in HSC than RGD or M6P alone. Further studies in vivo must prove whether this RGD-M6P can be applied as a cell-selective drug, targeting selectively the HSC [18].
- In addition to these ***protein-based carriers*** substituted with sugars or peptides, targeting to HSC was also achieved using antibodies, liposomes and viruses as carrier.
- In another study a ***human monoclonal antibody*** fragment is developed with affinity for synaptophysin, a protein expressed on the surface of HSC [19]. This was conjugated with a toxin to achieve cell-selective killing in HSC. This is the first report on the use of antibodies as a cell-specific drug carrier to resident hepatic cells.
- The liposomal research area also benefits from the new drug carriers to HSC. To ensure specific accumulation of ***liposomes*** within HSC in the fibrotic liver, in an experiment M6P-albumin is attached to liposomes [20-23], and in one liposomes substituted with the collagen type VI receptor recognizing peptide were used[24]. Efficacy studies in animal models showed an enhanced effect of hepatocyte growth factor on liver fibrogenesis when this growth factor was delivered into HSC using RGD-based liposomes.
- Another ***lipid*** that was reported to accumulate in HSC was ***cholesterol***. Oligonucleotides conjugated with cholesterol induced increased hepatic accumulation after systemic administration and HSC were found to be the major site of uptake (35% of the dose).

Gene Delivery to HSC

- Not only drugs but also genes are targeted to HSC.In most of the studies on gene delivery to HSC, adenoviral vectors are used. However, adenoviruses predominantly transduce hepatocytes due to their CAR expression.
- Internalization of adenoviral particles is additionally promoted by integrin present on cell membranes. HSC and myofibroblasts express high levels of various types of integrin, which may account for the adenoviral-mediated transfections of HSC.[25, 26].
- Other examples of adenoviral gene delivery as an experimental approach to treat liver fibrosis are the hepatic delivery of telomerase RNA by of PPAR-gamma, interference with TGFβ activities, and MMP gene delivery [27]. However, to achieve specific expression of genes into HSC using adenoviral vectors, virus re-targeting strategies need to be developed.

Retargeting can be achieved at three levels:

- adenoviruses can be conjugated with re-targeting moieties to induce binding to another receptor and avoid binding to the CAR receptor
- expression of adenoviral transgenes can be limited to target tissue by the introduction of cell-specific promoters, or
- The transgene product can be secreted locally and thereby delivered to neighbouring cells in the diseased area [28].

An example of the first is reported in which adenovirus is re-directed to PDGFβ- receptor using PDGF-receptor binding peptides.

- An example of the second retargeting strategy was there in which specificity of adenoviral transfection is improved by using a promoter in the viral construct which is only present in activated HSC? [29].It was demonstrated that all of these promoters were effective in producing a strong or partially selective expression in HSC *in vitro*. However, none of these promoters was able to create a specific or inducible expression of transgenes in these cells HSC *in vivo.*
- To date, mostly adenoviral vectors are applied to achieve transfection in HSC, and in these cases the HSC is just one of the cells that take up the vector. In general, the field of HSC targeting has seen much progress in recent years and most progress has been made with new protein-based drug carriers [12].

Therapeutic Approaches to Inhibit HSC Activation

Antioxidants

- ***Acetaldehyde*** generated from alcohol metabolism is responsible for both hepatocellular injury and HSC activation in alcoholic liver disease. Other ROS generated in patients with alcoholic liver disease (ALD) include H_2O_2 and hydroxyl ethyl radicals. These radicals are responsible for the apoptosis of hepatocytes exposed to ethanol *in vitro*.
- Adducts of these reactive metabolites and free radicals are immunogenic and may be the auto antigens that participate in the pathogenesis of chronic ALD. Therefore, antioxidative agents appear to be a good choice for preventing the hepatocellular injury and for attenuating the fibro genesis in ALD.
- ***Silymarin*** has been shown to be beneficial in inhibiting liver injury and in lessening the accumulation of hepatic collagen in advanced biliary fibrosis.However, a double-blind clinical trial showed that treatment of alcoholics with silymarin at a daily dose of 450 mg for 2 years did not improve the survival of the patients with advanced cirrhosis when compared with a placebo, and the treatment did not change the course of the disease.
- Nonetheless, it is argued that, whereas improvement of liver functions and reduction of ECM deposition in an early stage of cirrhosis or fibrosis may not necessarily change the survival rate, the evaluation of other parameters may indicate the clinical efficacy of the treatment in alcoholics.

- ***S-adenosyl-L-methionine(SAMe)***is a substrate of glutathione (GSH) synthesis and a donor of methyl groups which are involved in the metabolism of several amino acids in the body. It is thought that SAMe is crucial for the maintenance of normal biochemical functions in the nervous system and in the liver.
- It has been seen that SAMe protected isolated rat hepatocytes from the toxicity of bromobenzene and d-galactosamine and replenished glutathione levels in hepatotoxin-treated cells. Subsequently it

was shown that SAMe attenuated hepatic fibrosis induced *in vivo* by CCl_4 toxicity or bile duct ligation, and that SAMe was also useful in alcohol-induced fibrosis in baboons.

- Although the role of glutathione in HSC activation is not well defined, SAMe treatment will increase GSH levels in hepatocytes, which are often depleted in patients with ALD. The treatment probably will affect GSH levels in HSC too.
- ***Pentoxifylline*** an analogue of the methyl xanthine theobromine, is clinically useful for the treatment of conditions involving defective regional microcirculation. It was employed to inhibit the transition of HSC to myofibroblast-like cells (activated HSC) and to inhibit the proliferation of the cells *in vitro*. A study showed that pentoxifylline inhibited LPS-stimulated TNF-α production in monocytes, nuclear factor-kβ (NF-kβ) activation in HSC *in vitro*, and activated HSC *in vivo* (isolated from CCl_4-treated rats). Pentoxifylline also inhibited HSC proliferation and activation *in vitro*, which implies a direct effect on the cells.

- ***Polyenylphosphatidylcholine (PPC)*** is a mixture of polyunsaturated phosphatidylcholine (PC) rich in dilinoleoyl PC. PPC not only corrected ethanol-induced phosphatidylcholine depletion but it also prevented septal fibrosis and cirrhosis in baboons fed adequate diets. PPC treatment also led to a reduced number of HSC in patients with alcoholic disease. *In-vitro* experiments showed that PPC attenuated the transformation of stellate cells to myofibroblast-like cells, and inhibited PDGF-induced proliferation in rat HSC. PPC also acts on Kupffer cells by reducing LPS-stimulated TNF- α release and by enhancing interleukin-1β (IL-1β) secretion, which exerts an opposing action on TNF-α effect.

Blocking Cell-Matrix Interaction

- Integrins are a group of protein molecules on the cell surface that play a significant role in the mediation of ECM and HSC interaction and in the contraction of connective tissue (α1β1 integrin). Integrins serve as a bridge or "receptor" for cell interaction with the ECM though the adhesion activation of focal adhesion kinase (FAK), by increasing the levels of tyrosine phosphorylation in the cells. A recent study showed that P21 Ras, a member of a family of proteins that induce cell proliferation, cytoskeleton organization, and cell motility, operates as a protein-linking PDGF receptor to FAK in human HSC. Using soluble Arg-Gly-Asp peptides diminished the adhesion-induced tyrosine phosphorylation of FAK and inhibited HSC activation in vitro, as well as in a rat model of hepatic fibrosis induced by thioacetamide.

Herbal medicine

- Chinese herbal recipes display unique features in the treatment of acute and chronic liver injury. Varieties of recipes or herbal extracts, such as Xiao Caihu Tang Sho-aiko-to in Japanese), Recipe 861, glycyrrhizin, or silymarin (milk thistle), have been shown to be effective in the prevention and treatment of liver injury and fibrosis. Some of them were even found to be able to "reverse" the fibrotic liver to a nearly normal histology in patients with hepatitis B viral infection. Thus, the use of herbal medicines is providing a new approach in the treatment of liver disease.
- We have previously reported that ***glycyrrhizin***, an extract of liquorice, abrogated ethanol plus CCl_4-induced hepatic fibrogenesis in rats. It reduced liver injury and NF-kβ binding activity, as well as the degree of the liver fibrosis. Therefore, its effects are also thought to be secondary to its antioxidative properties. It has also been used for the treatment of viral hepatitis B because it was found that glycyrrhizin and related compounds could inhibit the release of hepatitis B surface antigen (HBsAg) from infected hepatocytes. They appear to function at multiple phases of the process of hepatic fibrogenesis. Many groups are presently interested in analysing the effectiveness of these agents [16].

CONCLUSION

In conclusion, we know that nearly all resident hepatic cells can be reached nowusing different drug delivery systems. To prevent that drug targeting is viewed upon as a complex way to deliver a drug to an organ that takes up most drugs anyhow, it is now essential to demonstrate the benefits of cell-selective drugs *in vivo* relative to untargeted drugs. The incidence of many liver diseases is increasing, in contrast to most other diseases in the western world. So, despite the fact that this organ is readily accessible by all drugs, no pharmacotherapy is available for most

liver diseases. We may therefore need a new strategy to treat hepatic diseases and cell-specific delivery of drugs may represent such a novel strategy.

REFERENCES

[1] Bataller R, Brenner D.A. Liver fibrosis. J. Clin. Invest 2005; 115:209-218.

[2] Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis G.M.M, Meijer ,D.K.F, Poelstra K. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatol. 1999; 29:1486-1493.

[3] Bataller R, Brenner D.A. Hepatic stellate cells as a target for the treatment of liver fibrosis. Semin. Liver Dis. 21:437-451.

[4] Friedman S.L. The virtuosity of hepatic stellate cells. Gastroenterology. 117:1244-1246

[5] Friedman S.L. Liver fibrosis – from bench to bedside. J. Hepatol. 2003; 38 Suppl 1:S38-S53.

[6] Neff et al The Current Economic Burden of Cirrhosis. Gastroenterology & Hepatology. 2011; 7.

[7] Friedman S L. Hepatic Stellate Cells: Protean, Multifunctional, and Enigmatic Cells of the Liver. Physiol. 2008; Rev 88: 125–172.

[8] Bal W, Christodoulou J, Sadler P J, Tucker A. Multi-metal binding site of serum albumin. J. Inorg. Biochem. 1998;70:33-39.

[9] De Bleser ,P J Jannes P, Van Buul- Offers, S C Hoogerbrugge, C M Van Schravendijk, C F H Niki, T Rogiers, V Van den Brande, J L Wisse, E Geerts. A Insulin like growth factor-II/mannose 6-phosphate receptor is expressed on CCl4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-β in cocultures with sinusoidal endothelial cells. Hepatol. 1995;21:1429-1437.

[10] Moreno M, Gonzalo T, Kok RJ, et al. Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats. Hepatology. 2010; 51:942-52.

[11] Beljaars L, Olinga P, Molema G, et al. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P (28)-HSA). Liver 2001; 21:320-8.

[12] K. Poelstra et al. Drug targeting to the diseased liver. Journal of Controlled Release 2012;161:188–197.

[13] Zhipeng et al. Novel materials which possess the ability to target liver cells. http://www.paper.edu.cn.

[14] Beljaars et al. Successful Targeting to Rat Hepatic Stellate cells using albumin modified with cyclic Peptides That Recognize the Collagen Type VI Receptor. J. Biol. Chem. 2000; 275 (17):.

[15] Beljaar L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis G M M, Meijer D K F, Poelstra K. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatol 1999;29:1486-1493.

[16] J. Wu and M.A. Zern: HSC: target for liver fibrosis treatment. J Gastroenterol 2000; 35:665–672.

[17] Y Sato, K Murase, J Kato, M Kobune, T Sato, Y Kawano, R Takimoto, K Takada, K Miyanishi, T Matsunaga, T Takayama, Y Niitsu. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone, Nat. Biotechnol. 2008; 26: 431–442.

[18] L S Wang, Y W Chen, D G. Li, H M Lu.Arg-gly-asp-mannose-6-phosphate inhibits activation and proliferation of hepatic stellate cells in vitro, World J.Gastroenterol. 2006; 12: 1303–1307.

[19] L J Elrick, V Leel, M G Blaylock, L Duncan, M R Drever, G Strachan, K A Charlton, M Koruth, A J Porter, M C Wright. Generation of a monoclonal human single chain antibody fragment to hepatic stellate cells — a potential mechanism for targeting liver anti-fibrotic therapeutics. J. Hepatol. 2005; 42: 888–896.

[20] J E Adrian, K Poelstra, G L Scherphof, G Molema, D K F Meijer, C Reker-Smit,H W M Morselt, J A A M Kamps. Interaction of targeted liposomes with primary cultured hepatic stellate cells: involvement of multiple receptor systems. J. Hepatol. 2006; 44: 560–567.

[21] J E Adrian, K Poelstra, G L Scherphof, D K F Meijer, A M. van Loenen-Weemaes, C Reker-Smit, H W M Morselt, P Zwiers, J A A M Kamps. Effects of a new bioactive lipid-based drug carrier on cultured hepatic stellate cells and liver fibrosis in bile duct-

ligated rats. J. Pharmacol. Exp. Ther. 2007; 321: 536–543.

[22] J E Adrian, J A A M Kamps, G L Scherphof, D K F Meijer, A M van Loenen- Weemaes, C Reker-Smit, P Terpstra, K Poelstra. A novel lipid-based drug carrier targeted to the non-parenchymal cells, including hepatic stellate cells, in the fibrotic livers of bile duct ligated rats. Biochem. Biophys. Acta-Biomembr. 2007; 1768: 1430–1439.

[23] J E Adrian, J A A M Kamps, K Poelstra, G L Scherphof, D K F Meijer, Y Kaneda. Delivery of viral vectors to hepatic stellate cells in fibrotic livers using HVJ envelopes fused with targeted liposomes. J. Drug Target. 2007; 15: 75–82.

[24] S L Du, H Pan, W Y Lu, J Wang, J Wu, J Y Wang. Cyclic Arg-Gly-Asp peptidelabeled liposomes for targeting drug therapy of hepatic fibrosis in rats. J. Pharmacol. Exp. Ther. 2007; 322; 560–568.

[25] Q Yu, R Shao, H S Qian, S E George, D C Rockey. Gene transfer of the neuronal NO synthase isoform to cirrhotic rat liver ameliorates portal hypertension. J.Clin. Invest. 2000;105: 741–748.

[26] Q Yu, L G Que, D C Rockey.Adenovirus-mediated gene transfer to nonparenchymal cells in normal and injured liver, Am. J. Physiol. Gastrointest. Liver Physiol. 2002; 282: G565–G572.

[27] K L Rudolph, S Chang, M Millard, N Schreiber-Agus, R A DePinho. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. Science 2000; 287: 1253–1258.

[28] M H Schoemaker, M G Rots, L Beljaars, A Y Ypma, P L M Jansen, H J Haisma, K Poelstra, H Moshage. Redirection of PDGF-receptor targeted adenovirusfrom hepatocytes to activated stellate cells. Hepatology 2003; 38 (4, Suppl. 1): 224A.

[29] J Herrmann, M Arias, E Van de Leur, A M Gressner, R Weiskirchen. CSRP2, TIMP-1, and SM22α promoter fragments direct hepatic stellate cell-specific transgene expression *in vitro*, but not *in vivo*. Liver Int. 2004; 24: 69–79.

[30] S Lotersztajn, B Julien. HEPATIC FIBROSIS: Molecular Mechanisms andDrug Targets. Annu. Rev. Pharmacol. Toxicol. 2005; 45:605–28.