Role of Serin/ Threonine Kinase Inhibitor in Therapy Non-Alcoholic Steatohepatitis and Alcoholic Hepatitis

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ABSTRACT
Liver fibrosis is a reversible response to a wound healing with marked accumulation of extracellular matrix which caused by injury to the liver. Liver fibrosis can be caused by various factors including alcohol and non-alcohol steatohepatitis. The process of fibrosis serves to localize the inflammation during chronic exposure. The hepatic stem cell (HSC) has a key role in the pathogenesis of liver fibrosis. The HSC activation is characterized by increased profibrogenic mediators including members of the TGF-β superfamily. In order to enable signal transduction, the mediator needs to bind to its receptors. The serine/ threonine kinase receptor is a receptor that binds to the TGF-β superfamily ligand, including TGF-β, BMP, activin and other mediators. The ligand receptor-binding activity will stimulate signal transduction that will translocate into the nucleus and phosphorylate various transcription factors that play a role in cell proliferation, differentiation, or apoptosis. There is currently no standard therapy for liver fibrosis. Based on the central role of the serine/ threonine kinase receptor in the pathogenesis of liver fibrosis, it is thought that the use of serine/ threonine kinase inhibitor is a promising therapy.

1. Introduction
Liver fibrosis is an excessive accumulation of extracellular matrix including collagen tissue in various chronic liver diseases. The process of fibrosis is closely related to a wound healing, and it serves to localize the inflammation during chronic exposure. In acute liver injury, the fibrosis process is transient, but in chronic injury, the accumulation of extracellular matrix will increase and the liver parenchyma will form a scar tissue progressively.

Without an adequate treatment, liver fibrosis may progress to liver cirrhosis and hepatocellular carcinoma. Cirrhosis is the end stage of liver fibrosis, characterized by the formation of nodules in the liver. This aberration is accompanied by impaired hepatic blood flow, leading to an increase in portal venous pressure. Portal hypertension is a major cause of mortality and morbidity in liver cirrhosis, characterized by the formation of ascites and esophageal varices. Nodular hyperplasia formed in cirrhosis, may develop into multiple adenomas, and multiple carcinomas, called hepatocellular carcinoma.

The progression of fibrosis to cirrhosis can occur within 15-20 years.

2. Liver Fibrosis
The liver parenchyma is composed of epithelial cells (hepatocytes), endothelial cells and non-parenchymal cells, the stellate hepatic cells (HSC) and Kupffer cells. Liver vascularization is derived from a sinusoid which has a microvascular structure. Sinusoids have endothelial walls that separated from hepatocytes with subendothelial spaces which are the site of HSC.

HSC has a key role in the pathogenesis of liver fibrosis. HSC was previously known as the Ito cell, liposit or perisinusoidal cell that known to be liver cells that produce collagen. These cells are present in the Disseperisinusoid space, which is the subendothelial spaces between the hepatocytes and endothelial cells. In a normal liver, HSC is a quiescence cell and serves as a retinoid deposit and maintains a basement membrane matrix.
HSC activation shows the conversion of a retinoid-rich cell that is resting into a proliferating, fibrogenic and contractile cell. Although the other cell populations may contribute to the accumulation of extracellular matrix, HSC activation is the most dominant mechanism in the liver fibrosis.\(^5\) HSC activation involves two main processes that are initiation and perpetuation. If the injury subsides, there will be a resolution process.\(^6\)

The initiation process is an early stage of hepatic injury, that characterized by an inflammatory response and oxidative stress, leading to changes in gene expression and phenotype. The perpetuation process is the stage to maintain an active phenotype. The activated hepatic stellate cells form the miofibroblasts, characterized by loss of retinoid retention, increased secretion of fibrogenesis mediators, increased \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) expression, and increased proliferation and contractility. Miofibroblasts will do the proliferation in response to various cytokines and growth factors. Proliferation of miofibroblast will be followed by an accumulation of extracellular matrix. Miofibroblasts may be inactivated or being apoptosis progressively if etiology is eliminated.\(^6,7,8\)

The stimulation of HSC activation comes from paracrine stimulation by injured hepatocyte cells, kupffer cell, endothelial cells and platelets. The stimulus includes various mediators, including TGF-\(\beta\), BMP, PDGF, VEGF, MMP and TIMP. The stimulus causes a quiescence HSC to become active.\(^9,10\)

In active HSC, the synthesis of endogenous TGF-\(\beta\) also increased significantly. TGF-\(\beta\) is the main mediator that stimulates the quiet HSC to be active. HSC activation is the most dominant route in the course of liver fibrosis. The TGF-\(\beta\) mechanism will promoting the liver fibrosis by inhibiting the degradation of extracellular matrix by suppressing the Metalloprotein Matrix (MMP) and promoting its natural inhibitor Tissue Inhibitor of Metalloproteinase (TIMP). Furthermore, TGF-\(\beta\) will inducing the formation of miofibroblasts by the mechanism of Epithelial Mesenchymal Transformation (EMT) in epithelial cells. The third mechanism is to induce matrix production through Smad and non-Smad pathways.\(^11\)

The main causes of hepatic fibrosis include hepatitis B virus infection, hepatitis C, alcohol, Non Alcoholic Steatohepatitis (NASH), haematocromatosis, Wilson's disease, antitrypsin \(\alpha\)-1 deficiency, autoimmune (primary biliary cirrhosis), and liver intoxication (drugs or nutrients). The course of liver fibrosis disease is often asymptomatic, the morbidity and mortality will be occur after the stage of cirrhosis of the liver is reached. And, until now there is no standard treatment for liver fibrosis.

3. NASH (Non-Alcoholic Steato Hepatitis)
Non-alcoholic fatty liver disease (NAFLD) refers to the spectrum of diseases, that ranging from fatty liver to liver inflammation and fibrosis, called non-alcoholic steatohepatitis (NASH). Obesity and type II diabetes are strongly associated with the development of NAFLD. 10-15\% of steatosis patients will develop into NASH, and 20-25\% of NASH patients eventually develop into liver cirrhosis that significantly increases the risk for hepatocellular carcinoma.\(^12\)

The pathogenesis of NASH consists of two stages: the occurrence of normal liver steatosis, especially as a result of insulin resistance in the periphery, that causing adipose cells and muscle cells oxidize lipids, leading to the release of free fatty acids. Fatty acids are then absorbed by the liver. Then, the fat accumulation will be form as a triglycerides in the liver. The second process is steatohepatitis, in which the accumulation of fat causes inflammation that mediated by
oxidative stress and fibrosis process, mediated by the secretion of various cytokines.\textsuperscript{12,13}

4. Alcoholic Hepatitis
Accumulation of ethanol in the liver can cause three different pathological pathways, namely fatty liver (alcoholic steatosis), alcoholic hepatitis and cirrhosis. Alcoholic steatosis is the most common form of liver injury and reversible when alcohol consumption is stopped. More serious alcoholic liver disease includes alcoholic hepatitis characterized by persistent liver inflammation, and cirrhosis characterized by progressive liver fibrosis.\textsuperscript{14,15}

The process of liver fibrosis begins in the perivenular region and is affected by the amount of alcohol consumed. Perivenular fibrosis and fibronectin deposition occur in 40\% -60\% of patients who consume alcohol more than 40-80 g / day for approximately 25 years. Perivenular sclerosis is considered a risk factor for the development of liver injury from alcohol to fibrosis or cirrhosis. Alcohol-induced liver cirrhosis is a micronodular, but occasionally it can be mixed both amicronodular and macronodular.\textsuperscript{14}

Alcohol-induced liver damage leads to the production of cytokines that mediate proinflammatory and profibrogenic responses, including TGF-\(\beta\), TNF-alpha, and PDGF. TGF-\(\beta\) plays an important role in underlying alcohol-induced fibrosis because ethanol metabolism will increase TGF-\(\beta\) production, while hepatocyte cell, Kupffer cell and HSC apoptosis also produce TGF-\(\beta\), active TGF-\(\beta\) has been proven to induce fibrosis in transgenic mouse models, and TGF-beta will synergizes with alcohol in inducing oxidative stress thus increasing alcohol-induced liver damage.\textsuperscript{16,17}

The various mediators and signal transduction pathways involved in liver fibrosis have been described. In order to enable signal transduction, the mediator needs to bind to their receptors. Among these receptors, an enzyme family called serine/threonine kinase has been known to be involved in the activation and proliferation of hepatic stellate cells. The serine/threonine kinase receptor is a receptor that binds to the TGF-\(\beta\) superfamily ligand, including TGF-\(\beta\), BMP, and other mediators. Ligand-receptor binding activity has a wide function in various cell physiology processes including proliferation, differentiation and apoptosis. Abnormal activity will disrupt cellular homeostasis and cause fibrosis malignancy. Returning the ligand-receptor binding activity as in normal circumstances can be a potential therapeutic target.

5. Serine/Threonine Kinase Receptor
Human as multi-cellular organisms lives in complex environments with multiple signal transduction pathways that contribute to their life survival. Serine/threonine kinase is one of the important enzymes in the signal transduction process that play a role in cell proliferation, differentiation, and apoptosis. Serine/threonine is an enzyme that catalyzes the phosphorylation of serine and threonine residues from target proteins using ATP.\textsuperscript{18}

The serine/threonine kinase receptor is a single transmembrane protein that consisting of extracellular domains for binding to ligands and intracellular catalytic domains facing cytosol. The activation process of serine/threonine kinase receptor is triggered by dimerization of two monomers of serine/threonine kinase receptors in line with autophosphorylated in intracellular domains so that catalytic activity increases. As a consequence, it will be produce biochemical signals and activate intracellular signal transduction.\textsuperscript{19,20}

There are two classes of serine/threonine kinase receptors which are structurally similar homodimers. The thype II receptors act as a primary receptor that bind to
ligand, whereas the type I receptors act as transducers that will activate signals on the downstream. The type I will be activated after the ligand binds to type II and phosphorylates serine and threonine.\textsuperscript{20}

The serine/ threonine kinase receptor transduces signals from TGF-\(\beta\) superfamily. TGF-\(\beta\) superfamily includes a large number of structural and functional proteins such as Transforming Growth Factor-\(\beta\) (TGF-\(\beta\)), Bone morphogenetic protein (BMP), activinsinhbin, Growth Differentiation Factors (GDF), and Anti Muellerian Hormone (AMH). The serine/ threonine kinase receptor that has been known to play a role in liver fibrosis include TGF-\(\beta\) Receptor (T\(\beta\)R), BMP Receptor (BMPR), Activin Receptor (ActR).\textsuperscript{11,19}

The association between the serine/ threonine kinase receptor and its ligand in the form of TGF-\(\beta\) superfamily will primarily phosphorylate the Smad protein to mediate signal transduction to intracellular. The members of the Smad protein family can be classified into 3 groups based on their function. The first group consists of Smad1, Smad2, Smad3, Smad5, and Smad8 which are receptor-regulated Smad (R-Smad). The second group is Smad4 which is a common Smad (Co-Smad). The third group is Smad6 and Smad 7 which is the inhibitory of Smad (I-Smad). R-Smad will bind to the serine/ threonine kinase receptor which is attached to the membrane and activated through its kinase activity. Co-Smad will bind to the activated R-Smad to form a complex that translocates to the nucleus. I-Smad works in opposition to R-Smad as a signal transduction inhibitor of TGF-\(\beta\) superfamily.\textsuperscript{21}In addition to activate Smad, the bond between the serine/ threonine kinase receptor with the TGF-\(\beta\) superfamily ligand also activates the non-Smad pathway.

HSC activation is an important marker in the pathogenesis of liver fibrosis. The process of transdifferentiation of inactive HSCs to miofibroblasts that trigger extracellular matrix accumulation and scar formation is a main process in the course of the disease. Serine/ threonine kinase has a mitogenic potential that plays a role in its activation and proliferation.

1. TGF-\(\beta\) Receptors (T\(\beta\)R)

TGF-\(\beta\) Receptor is a serine/ threonine kinase receptor that binds to a TGF-\(\beta\) ligand, a cytokine of TGF-\(\beta\) superfamily that consists of more than 35 structurally related proteins. The protein bonds form a homodimers and after its activation, it will regulates many cellular responses such as proliferation and apoptosis, differentiation and migration. TGF-\(\beta\) The receptor consists of TGF-\(\beta\) type I receptor (Activin receptor-like kinase 5-ALK5) and type II.\textsuperscript{11}

The TGF-\(\beta\) signal starts from the binding of TGF-beta to its serine/ threonine kinase receptor ie TGF-\(\beta\) type I and type II receptors. TGF-\(\beta\) bound to TGF-\(\beta\) type II receptors will cause type II receptors phosphorylating serine residues and threonine to activate type I TGF-\(\beta\) receptor. Type I TGF-\(\beta\) receptor will then activate the Smad and non Smad pathways.\textsuperscript{11,22}

TGF-\(\beta\) consists of TGF-\(\beta\)1, TGF-\(\beta\)2, and TGF-\(\beta\)3 which will be observed in various fibrosis diseases. The release of TGF-\(\beta\) by hepatocytes undergoing necrosis due to liver injury is one of the earliest signals in HSC activation that results in HSC transdifferentiation being miofibroblasts. Signal transduction TGF-\(\beta\) will inhibits HSC apoptosis and induces HSC to synthesize extracellular matrix such as collagen and fibronectin massively. TGF-\(\beta\) will suppress the production of matrix degrading proteases (MMP) and increase the protease inhibitors such as Tissue Inhibitor of Metalloproteinase (TIMP).\textsuperscript{8}

TGF-\(\beta\) will be activating the TGF-\(\beta\) type I receptor and cause a phosphorylation
of Smad2 and Smad3. pSmad2 and pSmad3 will bind to Smad4 to translocate to the nucleus and control the transcription of gene expression. At liver, TGF-β signals will participate in the fibrogenic response through hepatic stellate cell activation so that the TGF-β signaling in HSC plays a role in fibrosis progression. In addition to activating Smad2 / 3, the TGF-β bond on the receptor also stimulates the non-Smad pathway, which activates mitogen-activating protein kinases (ERK, p38 and JNK), phosphatidylinositol 3 kinase (PI3K) / Akt and small GTPase. Transduction signals via the TGF-β pathway are the main mechanisms underlying liver fibrosis.

2. BMP Receptors
The signal transduction of Bone Morphogenetic Protein (BMP) is as complex as TGF-β. BMP Receptors consist of type I and II receptors. BMP receptor type I consists of BMPRIA (ALK3) to bind BMP and BMPRIB ligands (ALK6) to bind BMP and Growth Differentiation Factors (GDF) ligands. BMP was first identified as a protein that induces endochondral bone formation and cartilage. There are 15 BMPs as members of the BMPs subfamily. BMP2, BMP4, BMP 7, and BMP 9 are known to be involved in fibrosis of the liver. 

BMP signal starts from BMP binding with its serine/threonine kinase receptor ie BMP type I and type II receptor. BMPs that bind to BMPRII will cause type II receptors to phosphorylate serine and threonine residues at BMP type I receptor. The type I BMP receptor then becomes active and subsequently phosphorylates Smad 1, Smad 5, and smad 8. The activated Smad complex will translocate into nucleus and bind to the Smad-responsive element to begin the transcription process.

BMP will promote liver fibrosis through the same mechanism as TGF-β by inhibiting the degradation of extracellular matrix, inducing the formation of miofibroblasts, and inducing matrix production via Smad and non-Smad pathways. However, there are differences in their effect on HSC proliferation activity. BMP does not affect the proliferation of HSC, in contrast to TGF-β which affects HSC proliferation. It can be concluded that BMP signals work in activated HSCs in the transdifferentiation process and retain the form of miofibroblasts.

Signal transduction from Growth Differentiation Factors (GDF) also uses BMP receptors. There is an overexpression of GDF 15 in cases of chronic liver injury. This is because GDF 15 also induces the formation of an extracellular matrix by suppressing Matrix Metalloprotein (MMP) and promoting its natural inhibitor Tissue Inhibitor of Metalloproteinase (TIMP).

3. Activin Receptor (ActR)
Activin is a member of the TGF-β superfamily known to have activity to stimulate the production of Follicle Stimulating Hormone (FSH) in the pituitary gland. Activin is further known to have activity against proliferation and other cell differentiation. The activin receptor resembles a TGF-β receptor that after active phosphorylation of Smad2 and Smad3.

In chronic liver injury, activin binding of the receptor plays a role in inhibiting the proliferation of hepatocytes and causing apoptosis. Activin also plays a role in the formation of extracellular matrix and increases the proliferation of miofibroblasts.

Given the central role of binding of the serine/threonine kinase receptor with its ligand in intracellular signal transduction, deregulation of serine/threonine kinase activity may promote fibrosis, apoptosis, and malignancy. It also suggests that the return of serine/threonine kinase activity as in normal
tissue is a treatment approach that can be developed.

6. Serine/ Threonine Kinase Inhibitor

In a liver fibrosis, there is an increased expression of serine/threonine kinase receptorin activated HSC as a result of stimulus from TGF β superfamily. In the last of few decades, the development of inhibitor serine/threonine kinase receptors has been widely practiced. Initially the development of this drug is intended as an anti-tumor, but the use of such inhibitors for the treatment of non-malignant diseases, especially fibrosis showed satisfactory results. Based on the central role of TGF-β superfamily in pathogenesis of liver fibrosis, it is thought that the use of serine/threonine kinase inhibitors is a promising therapy.

Serine/threonine kinase inhibitors prevent and block vital pathways with targeted signal molecules that play an important role in the survival of a cell. The serine/threonine kinase inhibitor can translocate through the plasma membrane and interact with the cytosolic domain of the serine/threonine kinase receptor. The serine/threonine kinase inhibitor will inhibits the catalytic activity of the kinase domain by disrupting the binding of ATP or substrate binding.

1. TGF-β Receptor Inhibitor

The signal transduction pathway TGF-β1 / Smad is the main mechanism underlying liver fibrosis disease, therefore it can be a clear therapeutic target to prevent or treat liver fibrosis. Several experimental studies have identified a number of alternative approaches to block the action of TGF-β, including the use of dissolved TGFR2 (sTβRII.Fc), antisense TGF-β1 oligonucleotides, and TGFR1 kinase activity inhibitors. Of these alternatives, clinical studies have begun with the use of TGFR1 kinase inhibitors in patients with fibrosis.29

Galunisertib (LY2157299 monohydrate) is a small molecule that inhibits TGF-β receptor I receptors that specifically decrease levels of phosphorylation of SMAD2, thus inhibiting the Smad pathway. This inhibitory effect was performed on research using mouse and human animals, so it used to be teraupetikarger in fibrosis and cancer cells. Currently, Galunisertib is intensively studied as an antifibrotic and has completed phase II clinical trials. In clinical trials, the use of galunisertib can be used as a drug in hepatocellular carcinoma that does not respond to sorafenib.30,31

The mechanism of action of galunisertib that inhibits the receptor TGF-β receptor I is by inhibiting the phosphorylation of SMAD2. In an ex vivo study of TGF-β-induced liver-slice preparations, galunisertib was shown to decrease the expression of TGF-β and α-SMA mRNAs. α-SMA is known to be a marker of liver fibrosis. Galunisertib also proven to decrease the formation of extracellular matrix and collagen type I.

The antifibrotic effects of galunisertib have been shown by many experiments. In all animal models attempted for liver fibrosis, the use of galunisertib showed antifibrotic effects, among other models of experimental animals with CCl4 induction, coledocus duct ligation (BDL), dimethylNitrosamine(DMN), diethylNitrosamine (DEN), and thioacetamide (TAA).32

Other drugs are EW-7197 which provides anti-fibrotic effects with inhibition of the TGF-β Smad pathway and ROS signal transduction. EW-7197 also inhibits HSC activation and expression of α-SMA and collagen type I. These effects have been demonstrated both in vitro and in vivo including in animal models with CCl4 induction, choledocus duct ligation (BDL), and bleomycin induction. These data support
that EW-7197 efficiently reduces in vivo and in vitro fibrosis and may be a therapeutic option for the treatment of liver fibrosis. Currently EW-7197 is in phase II clinical trials.33

2. BMP Receptor Inhibitor

The development of TGF-β inhibitors has been done for a long time and some drugs have entered the clinical trial stage. However, the development of molecules that inhibit BMP activity has only been done in recent years. Dorsomorphin is known to inhibit binding activity to BMP receptor type I as well as blocking BMP activity in phosphorylation of 1/5/8 Smad. Dorsomorphin is also known to inhibit activin activity and affect the signal TGF-β. Currently Dorsomorphin is in phase 1 clinical trials for the treatment of solid tumors.24

Development of BMP receptor inhibitors is currently performed on 2-aminopyridine (K02288). K02288 is known to have more specific activity inhibiting BMP binding with its receptor so the receptor can not phosphorylate Smad 1, Smad 5, or Smad 8 proteins that play a role in inducing matrix in fibrosis process. This inhibitory activity does not affect signal transduction from other TGF-β superfamily.24

3. Activin Receptor Inhibitor

Inhibitory activity against receptor activin is still continuing. Selective inhibition has been obtained from Folastatin administration, gene therapy isolated from ovarian follicles. For the development of new drug use there is a non-selective effect, wherein Dorsomorphin which has inhibitory activity against activin receptors also inhibits BMP receptor. SB-43 in addition to inhibiting receptors activin also inhibits signal TGF-β.34

Liver fibrosis has a complex mechanism. The involvement of serine/threonine kinase has been known to its mechanism and its binding with the ligand mediates signal transduction that plays an important role in fibrosis to malignancy. The use of serine/threonine kinase inhibitors is a potential therapy considering there is no standard therapy for liver fibrosis. These inhibitors still require further study so that humans can using it as liver fibrosis therapy.

REFERENCES

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