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Review

The Autotaxin - Lysophosphatidic Acid Axis as a Novel Therapeutic Target for Liver Fibrosis

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Special Issue: Exploring Novel Treatment Options for Liver Fibrosis: Can Anti-Angiogenics Succeed ?

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Abstract

Chronic liver disease (CLD) affects millions of people worldwide each year. Upon chronic liver injury, a wound healing process ensues, leading to the accumulation of extracellular matrix (ECM) proteins. If the injury persists, this leads to liver fibrosis with excessive scarring of the liver and loss of liver function. Lysophosphatidic acid (LPA), a signaling molecule, has shown to be involved in various biological processes, including the wound healing process. Elevated plasma levels of LPA and its catalyst autotaxin (ATX) have been detected in patients with liver fibrosis, suggesting a possible role of this signaling pathway in the development of liver diseases. This review focuses on the recent progress in studies on the LPA-ATX pathway and its involvement in liver fibrosis. This also includes the potential roles of the LPA pathway and ATX as a therapeutic target in liver fibrosis. The structural, functional and biochemical properties of LPA and ATX are also discussed.

Keywords

Liver fibrosis; lysophosphatidic acid; autotaxin; targeted therapeutics

1. Introduction

Chronic liver disease (CLD) affects millions of people and causes approximately 2 million deaths worldwide each year [1]. The main etiologies of liver cirrhosis, a late stage of liver scarring, are hepatitis B and C infections, alcohol abuse, and non-alcoholic fatty liver disease (NAFLD). Cirrhosis was the twelfth overall leading cause of death in the United States in 2007, with costs exceeding billions of dollars [2]. Liver fibrosis is a complex fibrogenic and inflammatory process that represents an early step in the progression of liver cirrhosis. The process is characterized by excessive deposition of extracellular matrix (ECM) proteins rich in collagen I and collagen III within the liver, resulting in liver scarring, change in liver structure, and distorted liver function [3]. When liver fibrosis is left untreated, it develops into cirrhosis and portal hypertension, hepatic encephalopathy, and/or liver failure. The only treatment for liver cirrhosis currently is liver transplantation.

Liver injury induces an inflammatory response, triggering the recruitment of macrophages into the liver. These macrophages thereupon produce cytokines and chemokines, which induce the activation of hepatic stellate cells (HSCs) into ECM producing myofibroblasts. These myofibroblasts migrate to the site of injury where they acquire proliferative, migratory, and immunomodulatory properties, and secrete large amounts of ECM proteins [4]. The transition of HSCs into myofibroblasts is characterized by the upregulation of α-smooth muscle actin, desmin, and collagen type I [3, 5]. Besides HSCs, both portal fibroblasts and bone marrow derived collagen producing cells are also able to differentiate into myofibroblasts. The origin of myofibroblasts in the liver caused by various etiologies can therefore be different [6].

Several signaling pathways have an essential function in liver fibrosis. The complex interactions between these pathways, diverse cells, and organs contribute to the progression of liver fibrosis. Novel therapeutics have been focused on inhibition of liver fibrosis specific pathways [7-9]. One pathway gaining interest as an important factor in liver fibrosis is the lysophosphatidic acid (LPA) – autotaxin (ATX) signaling pathway, since elevated levels of LPA and ATX are found in fibrosis [10-14].

1.1 Lysophosphatidic Acid

LPA, a bioactive lipid mediator, is a structurally simple signaling molecule with a wide variety of biological functions, including cell migration, neurogenesis, smooth-muscle contraction, angiogenesis, platelet aggregation, and wound healing [15-18]. It is a specific type of water-soluble glycerophospholipid with a glycerol backbone linked to a phosphate head group and a fatty acyl chain. Within the molecule, diversity in the fatty acyl chain is often observed, meaning that LPA molecule structures are present in different forms and derived from multiple sources [19]. LPA is able to activate a large amount of signaling pathways through coupling to specific G protein-coupled receptors (GPCRs). The outcome of these signaling pathways strongly depends on the cellular environment and the type of LPA molecule [15]. To date, six specific receptors have been identified, LPA_1-LPA_6. These receptors can be further sub-divided into two sub-groups, based on their primary structures. LPA_1-LPA_3 belong to the endothelial differentiation gene (EDG) family,

while LPA_4-LPA_6 share characteristics with the P2Y receptor family (purinergic G protein-coupled receptors), which target nucleotides rather than lysophospholipids [20]. All receptors are coupled with at least one of four different G-proteins: $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha s}$ and $G_{\alpha 12/13}$. Table 1 summarizes these proteins together with their tissue distribution and functions.

Table 1 LPA receptors and their *in vivo* function.

NAME	Synonym	G family	Tissue distribution (human)	Function
LPA_1	EDG2	$G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$	Most major human tissues	Brain development [21], pulmonary fibrosis [22], renal fibrosis [23], cancer metastasis [24], neuropathic pain [25]
LPA_2	EDG4	$G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$	Testis, leukocytes, cancer cells	Protection against radiation [26], progression of colorectal cancer [27], smooth muscle cell migration [28]
LPA_3	EDG7	$G_{\alpha i/o}$, $G_{\alpha q/11}$	Pancreas, prostate, testis, heart	Embryo implantation [29], tumor progression [30]
LPA_4	P2Y9/GPR23	$G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$, $G_{\alpha s}$	?	Vascular network formation [31], inhibition of white adipose tissue expansion [32], inhibition of osteogenesis [33]
LPA_5	GPR92	$G_{\alpha i/o}$, $G_{\alpha q/11}$	Spleen	Neuropathic pain [34], stress fiber formation [35]
LPA_6	P2Y5	$G_{\alpha 12/13}$	Skin, hair follicles	Hair growth [36], lymphocyte transmigration [37]

1.2 LPA Signaling

The fatty acid chain in the LPA receptors determines its activation by LPA. Generally, LPAs with an unsaturated fatty acid are able to activate LPA receptors, surpassing LPAs with saturated fatty acids. In addition, some LPA molecules prefer specific LPA receptor subtypes. An overview of the LPA receptor expression pathways is provided in Figure 1**.** LPA activates several signaling pathways through which it regulates migration, proliferation, and survival of various different cells. LPA signaling through $G_{\alpha 12/13}$ controls cytoskeletal remodeling, cellular invasion, and migration by the activation of Rho pathway proteins. The main function of LPA-coupled protein $G_{\alpha q/11}$ is the regulation of Ca^{2+} homeostasis by way of phospholipase C (PLC). The activation of phosphatidylinositol 3-kinase (PI3K) is regulated through G-protein $G_{\alpha i/o}$. PI3K thereupon stimulates the activation of the Akt (Protein Kinase B) pathway, which plays a major role in promoting cellular growth and proliferation. PI3K activation also stimulates the activation of Rac and Ras, leading to increased cell migration and increased expression of genes related to proliferation and invasion. Ras is also activated independent of PI3K. $G_{\alpha s}$ activated by LPA activates adenylyl cyclase (AC), thereby increasing cAMP concentration [19]. Furthermore, LPA induces cell proliferation and actin stress fiber formation via integrin-dependent pathways in an inside-outside-in manner [38].

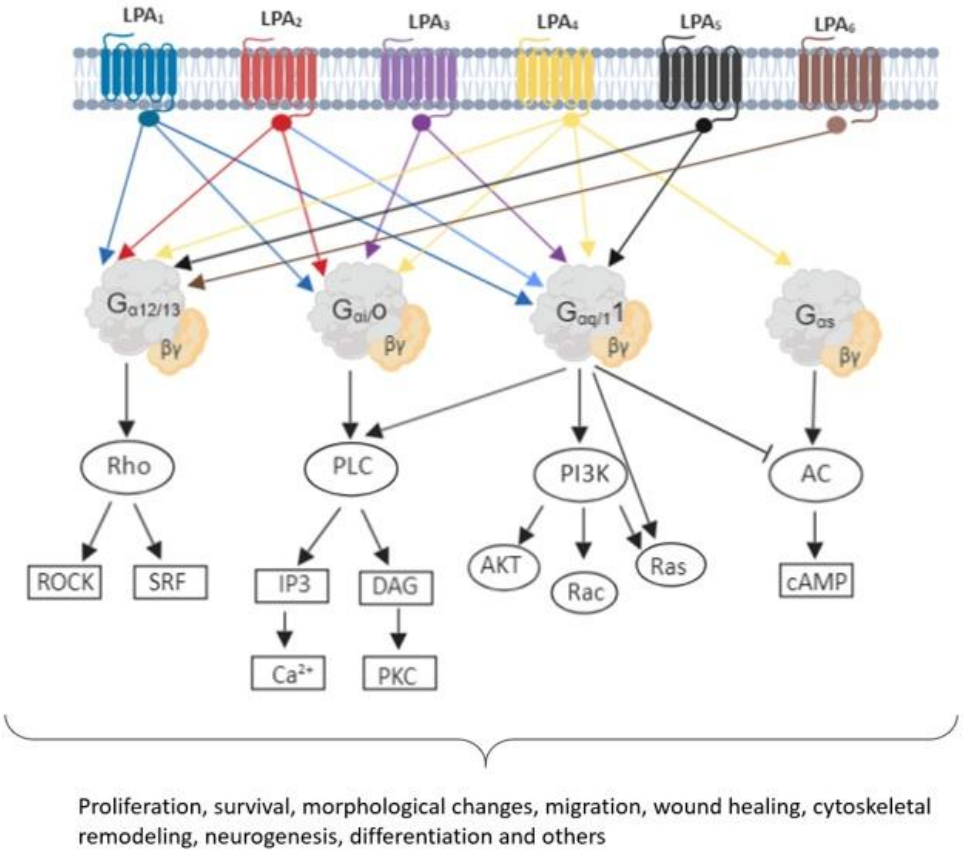

Figure 1 Summary of the intracellular lysophosphatidic acid pathway activated by the six lysophosphatidic acid receptors.

1.3 LPA Receptors and Their Localization

LPA$_1$ was the first receptor identified and was first located in the brain [19]. Expression of this receptor, however, can be seen throughout most major human tissues. Responses mediated by *LPA$_1$* include cell differentiation, proliferation, apoptosis and survival, regulation of neurodevelopment, and cell-cell contact [39]. Interestingly, *lpar1* expression in mice seems to be predominant in the nervous system [40]. *LPA$_2$* expression in human adults is detected in testis and leukocytes, while moderate expression in the spleen, thymus, pancreas, and prostate have also been observed [41]. Furthermore, deviant expression of *LPA$_2$* was observed in cancer cells, implying a possible role for this receptor in cancer [42]. This receptor is also thought to be important in the regulation of cell survival and cell migration in functions of the immune system and the nervous system [39, 43, 44]. Expression of *LPA$_3$* is most profound in the adult human pancreas, prostate, heart, and testis, and to some extent in the brain, lungs, and ovary [39]. The activation of physiological systems such as inflammation, Ca^{2+} homeostasis, and the male and female reproductive physiology are mediated through this receptor [45]. *LPA$_4$* only shares 20% homology with *LPA$_{1-3,}$* and it was the first LPA receptor identified in the P2Y family [46]. It can induce cell aggregation and cell adhesion, and the activation of LPA_4 was able to inhibit LPA-induced migration. *LPA$_4$* has the ability to negatively regulate cell motility and differential effects might be achieved by simultaneous expression of multiple LPA receptors [47, 48]. The receptor

LPA_5 mediates stress fiber formation, neurite retraction, and receptor internalization through its G-proteins. It is highly expressed in the spleen, and low levels are found in the heart, colon, placenta, and liver [19, 49, 50]. The most recently discovered LPA receptor is *LPA_6*. High expression levels have been found in human skin and hair follicles. The involvement of LPA_6 have been proposed in familiar hair loss [51, 52].

1.4 LPA Production by Autotaxin

The majority of the LPA in blood is synthesized from lysophosphatidylcholine (LPC), by the hydrolysis of its choline moiety. The enzyme responsible for this hydrolysis and therefore one of the main enzymes involved in LPA production is lysophospholipase D (lysoPLD) [53, 54]. Another source for the extracellular production of LPA is the direct hydrolysis of a fatty acid moiety from membrane-derived phosphatidic acid by LPA acyltransferase (Figure 2). The production of LPA is therefore not solely dependent on one specific pathway [53]. It was discovered that lysoPLD is functionally and structurally identical to ATX, an enzyme first discovered in the 1990s with implications in cancer cell motility [15, 53, 54]. ATX is expressed throughout various sites of the human body, and can be detected in plasma. The cellular source of ATX is still unknown, but it is likely being secreted by an array of cells that include platelets, adipose tissue, and high venule endothelial cells [55].

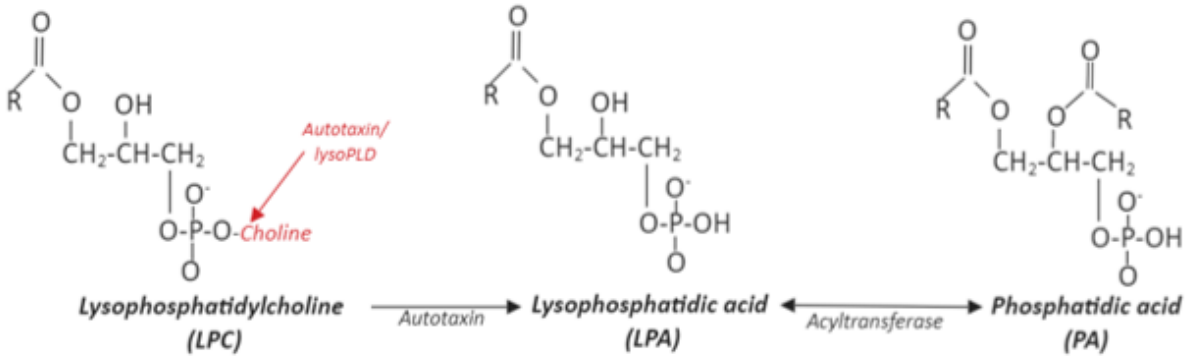

Figure 2 Extracellular production of lysophosphatidic acid is mostly dependent on two processes: the hydrolysis of a fatty acid moiety from phosphatidic acid (right) and the deletion of the choline moiety from lysophosphaticylcholine by autotaxin (left).

1.4.1 Structure and Function of ATX

ATX is a multifunctional and multimodular protein that possesses enzymatic lysoPLD activity and matricellular properties. ATX is a member of the ecto-nucleotide pyrophosphatases /phophodiesterases (ENPPs) and is also known as ENPP2. This group of enzymes is able to catalyze the hydrolysis of pyrophosphate or phosphodiester bonds in nucleotides. Looking at the structure of ATX, it has a central catalytic phosphodiesterase (PDE) domain, two catalytic somatomedin B (SMB)-like domains located at the N-terminus and a catalytically inactive nuclease (NUC) domain at the C-terminus (Figure 3) [50]. On each side of the PDE domain, there are linker regions connecting the SMB2 with the PDE domain (L1) and PDE with the NUC domain (L2, the lasso loop) [56]. The cysteine rich SMB-like domain contains an RGD tripeptide motif causing speculations on

its involvement in cell-ECM interactions. The catalytically inactive NUC domain might possibly be involved in substrate presentation and binding [56].

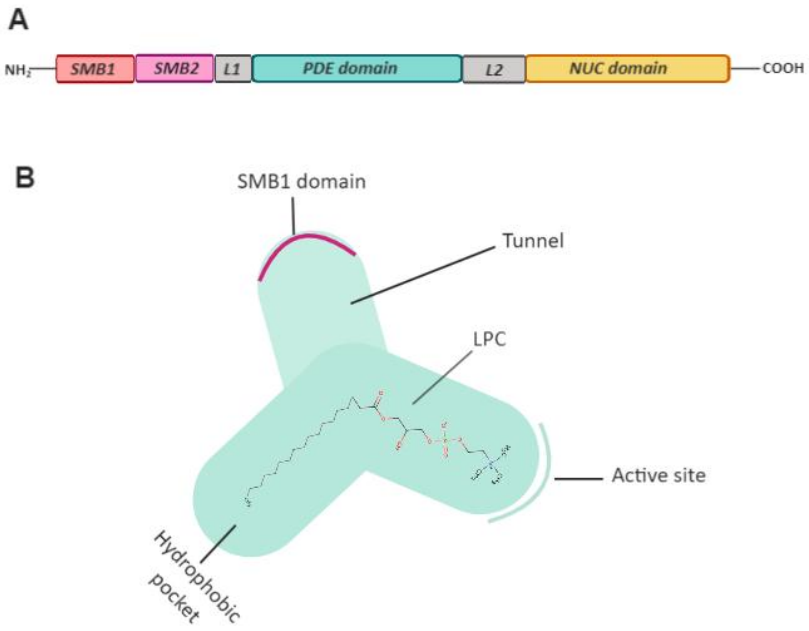

Figure 3 a) Different autotaxin domains, b) Schematic representation of the structure of autotaxin with the T-junction. In the hydrophobic pocket lysophophatidic acid and lysophosphatidylcholine molecules of various lengths and saturations can be stored.

The uniqueness of ATX within the ENPP family lies within the PDE domain and its interaction with the SMB-like domain. Because of the absence of an 18-amino acid sequence within this domain, it contains a deep hydrophobic, lipid binding pocket. This was thought to be an important evolutionary development, since this led to the ability of ATX to function as a lysoPLD. Furthermore, inclusion of this sequence into ATX led to a significant reduction in LPC hydrolysis, indicating that the absence of this sequence is important for LPA synthesis by ATX [57, 58].

Besides, a second LPA-binding site appears to be present in the form of an open tunnel (or channel), within the catalytic ATX domain. This 'T-junction' connects to the hydrophobic pocket and the catalytic site, and it is absent in the close relative of ATX, ENPP1, and also absent in bacterial NNP. This tunnel is partially hydrophobic, but at least one of the walls is hydrophilic. Therefore, it seems likely that this tunnel serves as an LPA exit channel, or as an entrance for LPC [50, 58]. However, the exact function of this tunnel remains elusive and requires further research.

1.5 LPA Pathway in Liver Fibrosis

The liver is a crucial organ in the human body, with over 500 essential functions that include detoxification, protein synthesis, the storage and release of glucose, and the production of essential biochemicals necessary for digestion. The liver therefore has developed a remarkable capacity to adapt to injury through tissue repair. Independent of the etiology (alcohol abuse, viral infection, drug abuse, etc.), a wound healing reaction ensues as a result of liver injury, resulting in the accumulation of ECM proteins within the liver. This progresses further, culminating in excessive tissue scarring and altered function, a process known as liver fibrosis.

HSCs are found to be pivotal features in liver fibrosis. Upon liver injury, they become activated and transdifferentiate into myofibroblasts, thereby acquiring scar-producing, proliferative, migratory, contractile immunomodulatory, and phagocytic properties [4]. LPA signaling has shown to induce a significant increase in the proliferation of HSCs and hepatocytes in culture [59]. LPA also enhanced HSCs mediated contraction, a key feature in the wound healing response associated with Rho-kinase activity [60].

Furthermore, elevated serum levels of LPA and especially ATX have been detected in vivo in liver fibrosis models in diverse recent studies [61, 62]. Hereby, specific studies were focused on different liver fibrosis origins including NAFLD [11] and chronic hepatitis B and C [12-14], demonstrating that elevated ATX levels are independent of disease etiology. A possible explanation on the serum ATX increase can be found in either or both of two aspects: an increase in ATX production as a result of fibrosis or a decrease in ATX clearance. In a healthy liver, ATX was shown to be rapidly taken up and degraded by the sinusoidal endothelial cells, indicating that it is largely removed from circulation after its first passage through the liver [63]. During the progression of liver fibrosis, the sinusoidal endothelial cells undergo phenotypic changes. These changes result into the capillarization of the sinusoids, leading to a restriction in the uptake of various substances, including ATX. This impairment in ATX clearance may account for the increased expression of ATX reported in fibrotic liver (Figure 4) [54, 64].

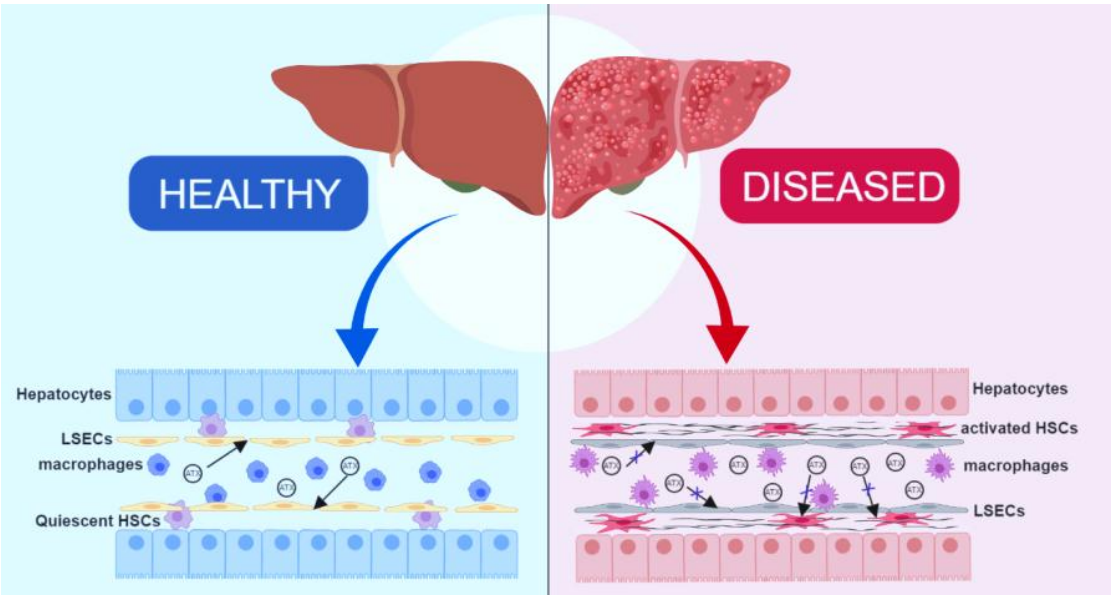

Figure 4 Possible mechanism leading to the Increased autotaxin levels in liver fibrosis. In healthy liver (left), autotaxin is taken up by the hepatic sinusoid and degraded in liver endothelial cells. In liver fibrosis (right) hepatic stellate cells are activated and cause the production of an excessive amount of ECM. This leads to phenotypic changes in the liver endothelial cells, causing capillarization of the sinusoids and thereby an impairment in the uptake of various substances, including autotaxin.

1.6 ATX as A Serum Marker for Fibrosis

As described previously, liver fibrosis is a result of a chronic liver injury, and may eventually progress into liver cirrhosis. Liver cirrhosis is irreversible, and can have major consequences

including portal hypertension, hepatocellular carcinoma, and liver failure. Early, reliable detection and stage prediction is extremely important. To date, the gold standard in evaluating the fibrotic stage of a patient is a liver biopsy. However, the need for non-invasive methods for the staging of fibrosis has increased throughout the years, since liver fibrosis is a dynamic process that has a significant reversal potential [65].

Serum ATX and plasma LPA levels both correlate with the histological changes detected in the different fibrotic stages. They therefore both have the potential for becoming non-invasive fibrotic markers. However, LPA is thermally unstable and is released from platelets which implies that it must be measured in plasma. ATX, on the other hand, is highly stable and can be measured in serum, which makes it more useful in a clinical laboratory [66]. Serum ATX has shown a significant correlation between ATX and liver fibrosis in patients with hepatitis B virus (HBV) infection. ATX was superior compared to other established non-invasive fibrosis markers: HA, WFA^{+}-M2BP, type IV collagen 7S, APRI, and FIB-4 [12]. Also, ATX was found to be a valuable marker of liver fibrosis in hepatitis C virus (HCV) infection. In a large study cohort with 593 patients, the clinical characteristics of ATX in HCV patients was investigated. This study verified the correlation between ATX serum levels and the liver fibrotic stage, validated by biopsy-determined histological scores in all patients. With this, they observed significantly higher serum ATX levels in women compared to men, in both the healthy controls and the patients with HCV. This indicates that gender difference is an important factor to account for [13]. In NAFLD patients, serum ATX levels were significantly related to the stage of fibrosis, and the ballooning score. However, the correlation between ATX and the fibrosis stage was comparatively weaker than that of patients with HCV [11]. Another study showed that ATX performed as a better fibrotic marker for patients with HBV, compared to hyaluronic acid (HA) and type IV collagen 7S, but the performance of ATX in NAFLD patients was poorer than HA and type IV collagen 7S. Interestingly, this study also evaluated patients with diabetes, rheumatoid arthritis, and cardiac dysfunction. With this, they showed that the elevated serum ATX levels were solely specific to liver fibrosis [67].

1.7 LPA/ATX Inhibitors for the Treatment of Fibrosis

To translate the direct relationship of the pathology of fibrosis and the LPA/ATX signaling pathway to possible treatment options, small molecule ATX inhibitors have been developed. To date, numerous ATX inhibitors are under development, of which most are analyzed in relation to idiopathic pulmonary fibrosis (IPF) rather than liver fibrosis. Of all these small molecule inhibitors, only one, GLPG1690, has entered clinical development [68]. Despite a substantial amount of data suggesting a direct role of LPA signaling in the pathogenesis of liver disease, very little data exists on the efficacy of ATX inhibitors in liver disease models. The ATX inhibitors tested in liver disease animal models are summarized in Table 2. Bain et al. evaluated the efficacy of small molecule ATX inhibitor PAT-505 in two different non-alcoholic steatohepatitis (NASH) mouse models, a choline deficient high fat diet (CDAHFD) mouse model and a high-fat diet combined with a subcutaneous injection of streptozotocin in stelic mouse animal model (STAM) [69]. In the STAM model, a reduction in the liver fibrosis area was observed after oral administration of PAT-505 (10 mg/kg) once a day; however, the results were not dose dependent, and therefore remained inconclusive. In the CDAHFD model, PAT-505 significantly reduced fibrosis development, without effects on hepatocyte ballooning, inflammation and steatosis. Evaluation of this inhibitor showed that it, in

contrast to many other ATX inhibitors, does not bind in a competitive manner. X-ray crystallography studies showed that PAT-505 binds in an allosteric site, while maintaining potent inhibition [69]. Another small molecule ATX inhibitor, Ex_31, was evaluated in two NASH models by M. Baader et al., [70]. The molecule was evaluated in a 10 week CCl_4-induced liver fibrosis model and in a more metabolically driven CDAHFD diet-induced 14 week model of liver injury. Surprisingly, researchers observed no signs of anti-inflammatory or anti-fibrotic efficacy of Ex_31 in the CCl_4 nor in the CDAHFD study. The explanation for this observation given by the authors is that LPA can be synthesized through ATX independent pathways, suggesting that ATX is not that important in the development of liver fibrosis [70].

Comparing the studies of the PAT-505 inhibitor to the Ex_31 inhibitor, it is important to note that while both studies used a similar NASH model, CDAHFD, the mouse models in the PAT-505 study were characterized with a lower fibrosis score than the models in the Ex_31 inhibitor study.

Table 2 Autotaxin inhibitors that have been tested in liver fibrosis animal models.

INHIBITOR	MODELS TESTED	EFFECTS FOUND	REFERENCES
PAT–505	CDAHFD, STAM	Significant reduction in fibrosis development; Mild reduction of fibrosis area	[69]
EX_31	CDAHFD, CCl_4	No anti-fibrotic effect; No anti-fibrotic effect	[70]

2. Concluding Remarks

CLD is major cause of death worldwide. The initiation of CLD, liver fibrosis, is a reversible process that allows treatment possibilities. Research into the exact mechanisms that drive liver fibrosis and the signaling pathways involved in liver fibrosis is therefore thriving. One of the signaling pathways with a potential role in the development of liver fibrosis is the LPA signaling pathway. LPA is involved in many important processes in the body, including the wound healing process. ATX, the major enzyme responsible for the production of LPA in human serum, was found to be upregulated in fibrosis. Thereupon, many studies have focused on the role of ATX in fibrosis in general, and liver fibrosis specifically. Hereby, researchers have found the possibility for ATX as a novel non-invasive early biomarker for the detection of liver fibrosis. Many studies have shown that the levels of ATX increase similarly to the staging of fibrosis. ATX would therefore be able to predict the fibrotic stage of the patient in a non-invasive manner. Furthermore, many small molecule ATX inhibitors have been under development, of which very little have been researched in relation to liver fibrosis. To date, only two have been used in animal studies, with contradicting results.

In summary, the LPA signaling pathway seems to play a significant role in liver fibrosis development. ATX has the potential of becoming an important biomarker in early detection and prognosis. LPA/ATX can be a promising therapeutic target for liver fibrosis; however, more studies are required to investigate novel small molecule inhibitors targeting LPA/ATX axis in liver diseases.

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Author Contributions

Richell Booijink wrote the paper. The work and the content was proposed by Ruchi Bansal. Ruchi Bansal provided the final comments and revised the paper.

Competing Interests

The authors have declared that no competing interests exist.

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