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Review Article

PDE4 INHIBITION : AN EMERGING THERAPEUTIC STRATEGY IN LIVER DISEASES

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Abstract

The world has been reported with 2 million mortalities a year due to liver disease and the major concern is unavailability of clinically approved treatment for liver diseases; therefore, there is an urgent need of novel treatments and targets. Cyclic adenosine monophosphate (cAMP, a second messenger) implicated in controlling of various cellular operations such as cell differentiation, inflammation, lipid metabolism by influencing gene expression and is the protective pathway. During liver injury cAMP is degraded by phosphodiesterases (PDEs), therefore cAMP pathway is widely focused in liver damage to evaluate its biological function as therapeutic target, based on the state of liver. Recently inhibition of cAMP specific PDE4 has been reported as a novel target as PDE4 inhibition raises intracellular cAMP level that consequently modulates inflammatory responses. Currently PDE4 inhibitors are presented as modulators of intracellular signals and gene transcription for the pharmacotherapy of liver disease. The present review summarizes the part of cAMP signaling pathway in liver well-being and ailments including non-alcoholic fatty liver disease as well as alcoholic liver disease. This review also discusses PDE4 inhibition as remarkable therapeutic approach for these conditions.

***Keywords*:** ALD (Alcohol liver disease), cAMP, Liver fibrosis, NAFLD (Non-alcohol liver disease), PDEs, PDE4 inhibitors.

Introduction

Liver is a major organ for controlling metabolic activities and maintaining homeostasis of whole energy in the body. Liver ailments such as alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are the foremost reasons for chronic liver illness. (Kmiec *et al*., 2001; Beavo *et al*., 2002; Masarone *et al*., 2014). Several mechanisms are involved for the progression of liver pathologies which includes parenchymal as well as non-parenchymal hepatic cells. (Kmiec *et al*., 2001). cGMP and cAMP are intracellular secondary messengers involved in various cellular functions and are regarded as protective pathways. These protective pathways become suppressed during liver injury by phosphodiesterase (PDE). (Beavo *et al*., 2002). PDE enzyme consists of 11 families (PDE1-PDE11), out of these PDE4 (cAMP-dependent) is present widely in circulatory tissues, smooth muscles, brain, liver and immune cells. (Keravis *et al*., 2012). PDE4 inhibition raises intracellular cAMP level that consequently modulates inflammatory responses and sustains immune balance. (Ahmad *et al*., 2015; Li *et al*., 2018). Therefore, PDE4 has been targeted as an efficient therapeutic approach for liver disease. With the increasing state of liver diseases, it has become seriously important to identify new pharmacological agents as FDA approved therapy still lacks behind for either NAFLD or ALD. Sequences of PDE4 inhibitors have shown their satisfactory therapeutic efficacy in regulating inflammation during liver injury. (Li *et al*., 2018). The current review summarizes the implication of cAMP-specific PDE4 pathway in the development of different liver disorders along with various PDE4 inhibitors developed till time and the novel PDE4 inhibitors that might be therapeutic agents for liver disease.

Structural and functional features of camp signaling and PDE4

The function of cAMP pathway is also associated with G-protein coupled receptor (GPCR), where GPCRs being the integral membrane protein responds to various extracellular stimuli (Pierce *et al*., 2002). Activation of GPCRs mediated by a certain ligand goes through a conformational modification that triggers G-protein complex (Gs) for the exchange of guanosine diphosphate for a guanosine triphosphate which is subsequently freed from complex. The released alpha subunit (Gs) attaches and stimulates adenylyl cyclase, which then catalyzes the translation of ATP into cAMP. Increased cAMP level promotes activation of several effector molecules; protein kinase A (PKA) and exchanged proteins made active by cAMP (EPAC) are the most typical and well renowned downstream targets (Cheng *et al*., 2008). Activation of PKA promotes phosphorylation of numerous downstream targets responsible for regulating variety of cell functions based on their locality. A typical PKA holoenzyme appears as tetramer consisting of two regulatory subunits bearing two catalytic subunits and cAMP binding domains. An increased cAMP level promotes two cAMP molecules to bind to each regulatory subunit which brings on a conformational alteration leading to the detachment of two regulatory subunits with the release of two catalytic subunits. The catalytic subunits once freed favors transfer of phosphate group from ATP to the substrate of protein containing serine or threonine residues that leads to an alteration in the activity of substrate (Taylor *et al*., 1990). Substrate of PKA carry out different cellular functions which involves rennin secretion in the kidney, dopamine signaling in the brain, kinases for smooth muscle contraction and glucose and lipid metabolism (Kawasaki *et al*., 1998). Earlier it was believed that PKA was

the mediator of cAMP and its major effects, later in 1998, EPAC was recognized as the new cAMP sensor protein (De Rooij *et al* 1998). EPAC exists in two isoforms, EPAC1 and EPAC2 both bearing cAMP binding domain similar to that of PKA. EPAC1 is distributed all over the body, whereas, EPAC2 is primarily articulated in adrenal glands, liver and pancreas. EPAC gets activated on binding with cAMP and further superfamily of Ras small GTPases stimulate Rap1 and Rap2. EPAC regulates various cellular roles ranging from cell adhesion to cardiomyocyte hypertrophy and adipocyte differentiation via EPAC/Rap1 signaling pathway. PDE4 is well recognized as a remarkable downs constituent of 5-hydroxytryptamine receptor, N-methyl-D-aspartic acid and β-adrenoceptor (Schippers *et al*., 2017). A-kinase-anchoring proteins (AKAPs) and receptors for activated C kinase 1 (RACK1, along with proteins containing SH3 domains are the proteins for interactions that influence function (intracellular) and localization of PDE4. (Jin *et al*., 2012). A group of enzymes recognized as cyclic nucleotide PDEs are responsible for the degradation of cyclic nucleotides. PDE4 has four subtypes, namely PDE4A-4D that are explicit for deprivation of cAMP instead of cGMP. (Wittmann *et al*., 2013; Kroegel *et al*., 2007). PDE4 family is broadly expressed and is responsible for majority of cAMP hydrolysis activity in cells. Inflammatory diseases Patients have raised appearance of PDE4 than healthy individuals. Inhibition of PDE4 leads to intracellular accumulation of cAMP and activation of cyclic nucleotide-gated ion channels, EPAC1/2 and PKA. These regulate the synthesis of anti-inflammatory and pro-inflammatory cytokines, neutrophil degranulation, T cells activation, epithelial integrity and performance of antigen-presentation via initiation of numerous downstream elements. (Li *et al*., 2018; Ravnskjaer *et al*., 2015).

cAMP SIGNALING IN LIVER DISEASES

The cAMP/PKA signaling pathway is well recognized as protective pathway in inflammatory diseases including liver diseases. Numerous studies have established role of cAMP/PKA in adaptable various metabolic functions such as lipid and carbohydrate via gene transcription and kinase activity. (Sutherland *et al.*, 1969). Production of cAMP is facilitated through establishment of adenylyl cyclase by hormones such as glucagon and adrenaline in liver. (Jitrapakdee *et al.*, 2012). cAMP increases glucose production via stimulating glucose producing pathways along with increased transcription of gluconeogenic enzymes. (Pilkis *et al.*, 1988). Moreover, phosphorylation of fructose-2, 6-bisphosphatase by PKA leads to activation and stimulation of enzyme. (Yajima *et al.*, 1999). Increased hepatic cAMP levels also inhibit lipogenesis via phosphorylation of mediators involved in the synthesis of fatty acids such as pyruvate dehydrogenase and acetyl-CoA carboxylase. (Lent *et al.*, 1983). Insulin has contrasting effects in comparison to glucagon and revere the phosphorylation of these enzymes stimulating lipogenesis and also decreasing glucose production. (Yajima *et al.*, 1999). cAMP regulates lipid metabolism via PKA mediated activation of transcription factors (cAMP) response element-binding proteins. (Mayr *et al.*, 2001). Numerous studies have investigated function of cAMP/CREB pathway in suppressing hepatic genes expression involved in the synthesis of lipid such as peroxisome-proliferator activator receptor (PPAR) γ, a nuclear hormone receptor which is a chief controller of lipogenic genes. (Herzig *et al.*, 2003). Activation of CREB also promotes the expression of PPARγ co-activator-1-alpha (PGC-1α) which then up regulates genes involved in mitochondrial fatty acid oxidation and gluconeogenesis. (Herzig *et al.*, 2001). Thus, cAMP signaling pathway emerges as an outstanding pharmacological target for liver diseases due to anti-fibrogenic, anti-lipogenic and anti-inflammatory property. For this the effect of numerous derivatives of cAMP, cAMP agonists and analogs or activators of EPAC and PKA has been studied in numerous diseases. (Ríos-Hoyo *et al.*, 2016; Sapio *et al.*, 2017).

Role of cAMP and PDE4 in ALD

Numerous studies have demonstrated function of cAMP/PKA signaling pathway metabolism of glucose, lipid and carbohydrate in liver via gene regulation, transcription and kinase activity. (Ravnskjaer *et al.*, 2015). The synthesis of cAMP is mediated by hormones like adrenaline, glucagon that activates adenylyl cyclase, which is the main producer of cAMP. Increased cAMP leads to increased glucose production; while PKA is involved in enzyme phosphorylation for glycolysis process and it also restrains glycolgen synthase for glycogenesis correspondingly. (Sutherland *et al.*, 1969; Jitrapakdee *et al.*, 2012). Raised level of cAMP in hepatic tissue inhibits lipogenesis by PKA-mediated phosphorylation of fatty acid synthesis enzymes like pyruvate dehydrogenase and acetyl-CoA carboxylase. (Lent *et al.*, 1983; Wahlang *et al.*, 2018). Study carried on ALD rat model also revealed that intraperitoneal cAMP administration prevented fat accumulation in liver due to alcohol feeding. Effect of cAMP on alcoholic hepatitis was due to cAMP mediated up regulation of alcohol-metabolizing enzyme, CYP2E1. A defect in CREB activation accumulates fat in liver. Study conducted on rat model fed with chronic alcohol showed lower hepatic CREB levels because of reduced hepatic cAMP levels. (Wu *et al.*, 2016). Further it was demonstrated that decreased cAMP levels in liver was due to increased PDE4 expression which further decreased CPT1A expression. PKA agonist elevates the level of CPT1A in primary hepatocytes. Besides lipolysis and fatty acid oxidation, reduction in lipid drop*et al*ong with inhibition of the recruitment of lipases to lipid droplets have been demonstrated in alcoholic hepatocytes mediated by activation of PKA by non-alcohol exposed hepatocytes on β-adrenergic stimulation. (Avila *et al.*, 2016). Inhibition of PDE4 by rolipram considerably repaired hepatic injury and steatosis induced due to ethanol *in vivo* and *in vitro* model of ALD by increasing hepatic levels of cAMP and reducing PDE4 levels. (Wu *et al.*, 2016).

Role of cAMP and PDE4 in NAFLD

Function of cAMP signaling pathway was also explored in NAFLD because of distinct hepatic property. NAFLD primarily presents itself as simple steatosis which may develop into steoto-hepatitis. (Liu *et al.*, 2014). The decline in lipid production and the rise in lipid metabolism will both be useful for enhancing NAFLD. For eg, GLP-1 treatment in fatty mice has shown decreased accumulation of lipids and oxidative stress due to increased cAMP level stimulated by GLP-1 and genes concerned with fatty acid oxidation, along with the suppression of genes for *de novo* lipogenesis. (Svegliati, Baroni *et al.*, 2011). Moreover, GLP-1 induced increased expression of PPARα-dependent gene for β-

oxidation of fatty acid in mouse model of NASH by increasing AMP-activated protein kinase and activity of PKA. (Zhang *et al.*, 2015; Kisseleva *et al.*, 2008). Resveratrol improved hepatic steatosis via stimulation of cAMP pathway and PDE4 inhibition in a mouse model of NAFLD. Nutritional supplementation of compact form of coenzyme Q_{10} inhibited progression of type 2 diabetes and obesity by reducing hepatic expression of PDE4, increasing the levels of cAMP as well as β-oxidation of fatty acid through SIRT-1/PGC-1α/PPARα pathway. Thus, revealing cAMP as a positive mediator for attenuating obesity and steatosis in NAFLD and NASH. (Zhang *et al.*, 2015; Kisseleva *et al.*, 2008).

Role of cAMP and PDE4 in liver fibrosis and other liver diseases

The main mechanism for the development of hepatic fibrosis involves activation of hepatic stellate cells and modification of hepatic stellate cells (HSCs) into contractile, proliferative and chemotactic myofibroblasts. (Kisseleva *et al.*, 2008; Aoyama *et al.*, 2010; Kisseleva *et al.*, 2011). Liver injury manifests by migration and accumulation of excess amounts of extracellular matrix elements like fibronectin along with collagens. (Lopez-Sanchez *et al.*, 2014). Extreme deposition of scar affects liver function by altering blood flow that ultimately leads to liver failure. (Lopez-Sanchez *et al.*, 2014; Yang *et al.*, 2016). The cAMP signaling pathway displays a significant role in inhibiting fibrogenic pathways in hepatic stellate cells, thereby decreasing HSC activation. (Schippers *et al.*, 2017). The beneficial effect of cAMP pathway is associated with PKA activation. A study evaluated the role of cAMP/PKA and cAMP/EPAC pathways in acetaldehyde-induced hepatic stellate cell activation and it was found that acetaldehyde suppressed EPAC1 but increased the expression of EPAC2. cAMP analog, significantly decreased collagen synthesis along with hepatic stellate cells proliferation stimulated by acetaldehyde. (Schippers *et al.*, 2017; Kelava *et al.*, 2013). In addition, PKA activation increased the expression of HSC marker, collagen and alpha smooth muscle actin (αSMA). On the other hand, depletion of EPAC2 by siRNA suppressed HSC activation confirmed by reduced collagen and αSMA. (Arai *et al.*, 1995). Mouse model of carbon tetrachloride-stimulated fibrotic liver had reduced EPAC1 expression as compared to normal livers. (Cullen *et al.*, 2004). Similarly, decreased level of EPAC1 was found in fibrotic livers of human. Administration of cAMP activator such as prostaglandin E2 enhanced the levels of EPAC1, thus leading to the inhibition of proliferation and migration of stellate cells stimulated by platelet-derived growth factor (Schippers *et al.*, 2017). A study showed that an increased cAMP level provides protection against liver injury in model induced by drug acetaminophen (Kelava *et al.*, 2013). Moreover, cAMP analog, dibutyryl cAMP also proved to be beneficial in LPS-induced liver injury (Arai *et al.*, 1995). The effect of cAMP signaling was also studied in toxicity induced due to bile acid in hepatocytes (Cullen *et al.*, 2004; Webster *et al.*, 2002; Johnston *et al.*, 2011). Another mechanism of protection mediated by cAMP involves CD95 (FasR) phosphorylation by PKA that leads to inhibition of caspases, DISC and ultimately apoptosis. (Bhattacharjee *et al.*, 2012). Increased cAMP levels lead to increased inactivation of hepatic stellate cells and reduces fibrosis in rat model of cholestatic liver injury (Kawy *et al.*, 2015). cAMP/PKA defensive role was also observed in hepatocellular carcinoma mediated by phosphorylation of epidermal growth factor receptor by PKA, which further dephosphorylates signal transducer and activator of transcription 3 (STAT3) and suppresses STAT3 target genes; thus, promotes reticence of hepatocarcinogenesis (Zhou *et al.*, 2017). PDE4 inhibitor raised intracellular cAMP levels which then interfered with cell cycle progression and suppressed cell explosion in hepatocarcinoma-derived cell line (HepG2 cells) (Massimi *et al.*, 2017).

PDE4 inhibitors in liver diseases

The function of PDE inhibitors is to inhibit the degradation of cAMP and cGMP by the PDEs and prolong the actions of cAMP and cGMP signaling. Among cAMP-specific PDEs, PDE4 is targeted for inhibition as a therapeutic strategy. The outcome of PDE4 inhibition is evident and well recognized for neuroprotection, anti-inflammation, wakefulness and procognition. Rolipram is an exemplary PDE4 inhibitor earlier considered to be antidepressant during 1990s, but because of its narrow therapeutic window it was withdrawn (Zhu *et al.*, 2001). Despite of being withdrawn, rolipram is however utilized in research for evaluating the beneficial effects of PDE4 inhibition in numerous disorders such as spinal injury, lung injury, Alzheimer's' disease and auto-immune disease. Roflumilast, a PDE3/PDE4 inhibitor had displayed its bronchodilatory and anti-inflammatory properties in chronic respiratory diseases (Abbott-Banner *et al.*, 2014). Endotoxin (LPS) has shown its pathological role in ALD. In ALD, LPS induces TNFα production via activation of PDE4, particularly PDE4B (Jin *et al.*, 2005; Gobejishvili *et al.*, 2008). Rolipram and roflumilast have shown their protective results in different murine models (liver injury) (Jin *et al.*, 2002; Jin *et al.*, 2005; Gobejishvili *et al.*, 2008; Gobejishvili *et al.*, 2013). Both approaches either knockout or pharmacological inhibition of PDE4 have shown significant effect on hepatic steatosis provoked by alcohol by preventing the alcohol-induced decrease in hepatic Cpt1a expression *via* the PPARα/Sirt1/Pgc1α pathway *in vivo* (Avila *et al.*, 2016). Roflumilast enhanced glucose tolerance in mice fed with high-fat diet and reduced steatohepatitis via PDE4 inhibition. Roflumilast also lowered weight gain and increased energy expenditure. Several studies in human have also showed that treatment with roflumilast improved insulin resistance and reduced fat mass (Feng *et al.*, 2017). Pentoxifylline showed protective results adjacent to NASH led by methionine-choline deficient diet via suppressing TNFα manufacture and alleviating ER stress (Jensterle *et al.*, 2014; Chae *et al.*, 2012). Data obtained from a clinical study revealed that pentoxifylline in NAFLD reduced raised levels of enzymes of liver in patients. (Yalcin *et al.*, 2014).

Pentoxifylline also reduced HSC activation and development of fibrosis in *In vitro* and *in vitro* studies, revealing inhibition of PDE4 as helpful approach in liver fibrosis (Zeng *et al.*, 2014; Movassaghi *et al.*, 2013; Zhang *et al.*, 2012; Toda *et al.*, 2009). In activated LX-2 hepatic stellate cells, expression of TGF-β1 has been suppressed by pentoxifylline due to inhibition of pro-fibrogenic hedgehog signaling pathway (Li *et al.*, 2016). ASP9831-PDE4inhibitor was tested in clinical trial for NASH but levels of ALT/AST was not reduced, regardless of a definite mechanism of action (Ratziu *et al.*, 2014). Recently, roflumilast shown to reduce liver fibrosis induced by diethyl nitrosamine in rats by

modulating cAMP/CREB/TLR4 inflammatory and fibrogenic pathway (Essam *et al.*, 2019). PDE4 inhibition by clinically approved PDE4 inhibitors, roflumilast and rolipram in both *in vivo* and *in vitro* study significantly reduced hepatic steatosis and injury induced by ethanol via several mechanisms, involving reduced endoplasmic reticulum and oxidative stress (Rodriguez *et al.*, 2019). There exists problem in the development of PDE4 inhibitors for ALD and NASH and further efforts are needed to discover potential drug candidates. Recently researchers have designed PDE4 inhibitors for various inflammatory disorders that can be tested and evaluated for various liver diseases. 4, 5, 6, 7-tetrahydro-1*H*-1,2-diazepin-7-one analogs were designed and synthesized as PDE4 inhibitors for respiratory, inflammatory, metabolic and CNS disorders. (Guariento *et al.*, 2017). A new series of butanehydrazide derivatives of purine-2,6-dione was evaluated in rats with LPS-induced endotoxemia that displayed inhibition of cAMP-specific PDE4 and offered a strong anti-TNF-α effect (Chłoń-Rzepa *et al.*, 2018). A series of 5-dimethylpyrazole derivatives were synthesized and evaluated for the blockade of PDE4 and LPS-induced TNF*α* release (Hu *et al.*, 2018). Thus, an efficient and productive preclinical test of desired drug targeting PDE4 in liver disease is needed with accurate drug dosing or combined drug therapy.

Conclusion

Hepatic disorders have affected both developed and developing countries. Especially ALD and NAFLD have similar pathological features, developing steatosis, fibrosis and inflammation. Unavailability of FDA approved drugs has led researchers for identification of potential targets and drugs for liver diseases. cAMP signaling pathway regulates physiological features of liver function which is controlled by PDEs. Targeting these enzymes, particularly PDE4 with its inhibitor might become a suitable treatment for liver diseases. Based on preclinical studies PDE4 specific inhibitors have been demonstrated as a therapeutic potential for the treatment of liver diseases. However, extensive preclinical with clinical studies are still needed to discover efficacy of drugs in different stages of liver disease.

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Conflict of interest

The authors declare no conflict of interests.

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