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Review Article

Bile Acid Signaling and Cardioprotection

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Abstract: Bile acids (BA) are classically known as an agent important in lipid absorption and cholesterol metabolism. Nowadays, BAs have been found to be involved in various cellular signaling pathways such as protein kinase cascades, cyclic AMP (cAMP) synthesis and calcium mobilization. In addition, they have also been shown to regulate glucose and energy homeostasis. Bile acids are ligands for several nuclear hormone receptors, including FXR. Recently, muscarinic receptor and TGR5, G-protein-coupled receptor (GPCR), have been suggested to play a role in bile acid activity which is independent of nuclear hormone receptors. Moreover, BAs have also been studied in other GPCR associated pathways, namely sphingosine-1-posphate and glucagon receptor. Hydrophobic bile acids have been proven to affect heart rate and its contraction. Elevated bile acids are associated with arrhythmias in adults and fetal heart. Altered ratios of primary and secondary bile acid are reported in chronic heart failure patients. Meanwhile in patients with liver cirrhosis, cardiac dysfunction has been strongly linked to the increase of serum bile acid concentrations. In contrast, the most hydrophilic BA known as ursodeoxycholic acid has been found beneficial in improving peripheral blood flow in chronic heart failure patients and protecting heart against reperfusion injury.

Keywords: heart; ursodeoxycholic acid; cardioprotection; signaling

1. Introduction

Bile acids are derived from cholesterol and stored in the gall bladder. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the two most common types of primary bile acids, which are synthesized in the liver and conjugated to either taurine or glycine. The conjugated primary bile acids are known as the glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GDCA), and taurochenodeoxycholic acid (TDCA). These conjugated bile acids are known as bile salt. Bile acids pool consists of only 2.5 – 5.0 g in the liver [1]. Ninety-five percent of bile acids reabsorption occurs in the small intestine [2]. The most common secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), are synthesized by microbial flora of small intestine. Those two bile acids are produced as a result of deconjugation and dehydroxylation of primary bile acids. The cytotoxicity of bile acids is based on their structure formation. While their hydrophobicity is based on the number and position of its hydroxyl group in the ring structure (Table 1). High concentrated hydrophobic bile acids such as CDCA and DCA are associated with colon cancer, gallstones, and other gastrointestinal (GI) diseases [3]. LCA is the most hydrophobic bile acid and known to be insoluble throughout the small intestine. However, it is too little to be reabsorbed back into the enterohepatic circulation. Thus, the amount of LCA in the feces is higher. The least toxic bile acid called UDCA is synthesized by intestinal microflora by dehydroxylation of free bile acids, CDCA. UDCA is the most hydrophilic bile acid and little amount of it could be found in the feces.

Hydrophobicity	Types of bile acids
	Glycine-conjugated; GCA, GDCA, GCDCA
	Taurine-conjugated; TCA, TDCA,TCDCA
	Lithocholic acid (LCA)
	Deoxycholic acid (DCA)
	Chenodeoxycholic acid (CDCA)
	Cholic acid (CA)
	Ursodeoxycholic acid (UDCA)

Table 1: The bile acids hydrophobicity decrease with increase of OH groups.

Bile acids pool circulates in human through the enterohepatic circulation. This enterohepatic circulation involves liver as the main organ, gallbladder as a storage organ for the bile, and the small intestine to help in digestion of fats as well as excretion and reabsorption. Bile acids are synthesized in the liver through enzymatic reactions where the hydrophobic ring structure of the cholesterol is being digested into more water-soluble amphiphatic compounds [4]. Four types of transport are involved to help in bile acid reabsorption: active transport, passive ionic diffusion, passive non-ionic diffusion and passive micellar diffusion. Bile acids are absorbed through those transport processes and circulated back to the liver. Small quantities of bile acids are present in the systemic circulation and very small amount was excreted through urine and feces (300-600 mg/day) [5]. The insoluble fats are also removed together with 10-20 % of the bile through feces [6].

2. Bile Acid as a Signaling Molecule

Materials Bile acids are involved in lipid metabolism [7-8]. cholesterol elimination, bile flow, cholesterol biosynthesis, and glucose metabolism. Bile acids are not just a type of molecule that circulates in the body, but they have also been discovered to play an important role as a signaling molecule in cell proliferation and differentiation of cells such as hepatocytes [9]., gastric cancer cells [10] and colon cancer cells [11]. Studies have shown that the bile acid signaling mechanism involves a response which could be mediated by the nuclear receptor-mediated response (FXR) [12] or the membrane receptor-mediated response (TGR5) [13]. Muscarinic receptor and sphingosine-1-Phosphate (S1P) are the other types of receptor reported in transmitting the signal of bile acids.

2.2 Nuclear receptor-mediated response

Farnesoid X-receptor (FXR) is the prominent target for most bile acids. FXR ligand-binding domain of hydrophobic face binds to hydrophilic face of bile acids. The function of FXR receptor was firstly described in the gut to aid in the reabsorption of bile acids from intestine to portal system. FXR is known to be highly expressed in the liver, intestine, adipose tissue, pancreas and adrenals [14]. FXR was firstly reported to be expressed in normal vascular smooth muscle and atherosclerotic blood vessels of human by Bishop-Bailey et al. [15]. The group further reported that FXR regulates cells proliferation and expression of FXR target genes, small heterodimer partner (SHP) and the

phospholipid transfer protein (PLTP) in vascular smooth muscle cells [15]. The prominent FXR bile acid ligand is CDCA. In LCA-induced cholestasis model, CDCA activity was reduced and BSEP expression was downregulated which lead to decrease hepatic bile acid secretion and increasing bile acid concentration in the liver leads to liver damage [16]. This shows that FXR-activated BSEP expression by CDCA is crucial in protecting the liver. CDCA activated FXR expression in the intestine concurrently activates the intestinal acid-binding protein (IBABP) which mediates cholesterol secretion from the body [17]. Activation of FXR in cells would signal the cells to activate the related mechanism of lipid and glucose synthesis [18]. In the liver, the influx bile acid activates FXR and triggers CYP7A1 activation to synthesize cholesterol to bile acid [19]. CYP7A1 is rate limiting protein of bile acid metabolism. FXR is being activated most effectively by CDCA in comparison to DCA, LCA, and CA. Apart from the liver, in cholangiocytes; unconjugated CDCA inhibits growth of the tumor by upregulating the FXR expression [20]. Recently, Desai et al., [21] reported that excess bile acids leads to cardiomyopathy and heart dysfunction. The study reported that in FXR and SHP double knockout mice (model for cirrhosis), reduction in fatty acid metabolism was observed. Consequently, the mice developed cardiac dysfunction due to suppression of proliferator-activated receptor-γ co-activator 1α genes, a key regulator for fatty acid metabolism.

Bile acid has been shown to be important in lipid and glucose homeostasis. Activation of FXR in cells would signal the cells to activate the related mechanism of lipid and glucose synthesis. In addition, activated FXR allowed the bile flow through canaliculi. Apart from the FXR, other nuclear receptors involved in the regulation of lipid and glucose metabolism are Pregnane X-receptor (PXR) and Vitamin D receptor (VDR) [22]. PXR involves in the breakdown and elimination of BAs. PXR acts as a receptor for xenobiotics to detect secondary BAs metabolism. LCA stimulates PXR and subsequently activates drug resistance protein such as MDR1, MRP3, MRP2 and CYP3A for bile acid transport and detoxification [23]. FXR and PXR work in together in eliminating the effect of bile acid induced toxicity by downregulate the expression of cholesterol 7α hydroxylase (CYP7A1) [24].

Vitamin D receptor (VDR) is activated by its natural ligands, 1α, 25-dihydroxy-vitamin D3 [1α, 25 $(OH)_2$- D3] and lithocholic acid (LCA). The signaling effect of both ligands was studied by Han et al., [25] in primary human hepatocytes. The group reported that both ligands activate VDR-signaling pathway through the activation of ERK 1/2 pathway and further phosphorylates nuclear VDR to stimulate the inhibition of CYP7A1 gene transcription in human hepatocytes, thus protecting the cells from further damage in cholestatic liver injury [25]. In small intestine, LCA induced the CYP24a1 expression, VDR target gene, as efficient as the main natural ligand of VDR, 1α, 25 $(OH)_2$ [26]. Though, the effect is prominently observed in the ileum than jejunum and duodenum. Vitamin-D deficiency may increase the risk of inflammatory bowel disease, renin hypertension and cardiac hypertrophy [27]. VDR is known as an important nuclear receptor in regulating calcium homeostasis, immunity and cellular differentiations. Furthermore, VDR activation has been reported to be involved in BAs transport, metabolism, and detoxification through the stimulation of CYP3A [9]. A selective binding of LCA acetate to VDR was further discussed and proposed to be 30 times better than LCA itself and had less specificity binding with FXR and PXR [28].

Liver X receptor (LXR) is important in cholesterol homeostasis involving the bile acid synthesis, metabolism, transport, and excretion. LXR was activated by endogenous oxysterols and oxidised derivatives of cholesterols. Increase of oxysterols in cells induces LXR activation to protect the cells from the effect due to high cholesterol level. There are two isoforms of LXR known as LXRα and LXRß. Both isoforms have been reported to be involved in cardiovascular diseases and atherosclerosis reduction. Bradley et al., (2007) reported that the LXR activation reduces formation of atherosclerosis lesion [29]. Furthermore, the involvement of LXR in bile acid synthesis is through the LXR binding response elements (LXRE) on CYP7A1, which is the rate-limiting enzyme of the cholesterol pathway. It has been shown by Song et al., (2000) that taurine conjugated UDCA, TUDCA is able to activate the LXRE in the CYP7A1 promoter to induce LXR activation and promote CYP7A1 activity [30].

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2.3 Takeda G protein-coupled receptor 5 (TGR5)

TGR5 receptor (GP-BAR 1 or M-BAR) was first discovered by Kawamata et al., (2003). TGR5 expression is expressed in different types of cells such as endocrine glands, adipocytes, muscles, immune response, spinal cord, and the enteric nervous system. TGR5 has been reported to suppress rabbit alveolar macrophages function in response to bile acids (LCA, DCA and CDCA) treatment and subsequently inhibited lipopolysaccharide (LPS)-induced TNFα secretion [31]. They also show that the TGR5 activation by TLCA was found in the plasma membrane and cytoplasm of Chinese hamster ovary (CHO) cells. TGR5 is a type of GPCR that requires activated Gs-protein leading to cAMP accumulation [31] and upregulates kinases protein such as AKT and ERK 1/2. TGR5 found to be highly regulated in the intestine. TGR5 signaling regulates the intestinal glucagon-like peptide-1 (GLP-1) release and subsequently improved the liver function of obese mice [32]. GLP-1 involves in glucose homeostasis to increase the insulin secretion and improve glucose tolerance. TGR5 also protects liver injury through inhibition of LPS-induced cytokine expression in Kupffer cells [33]. TGR5-cAMP dependent pathway increases bile-duct ligation thus it is effective to treat obstructive cholestasis by preventing cytokine production. Recent study shows that bile acid receptor TGR5 deletion in mouse macrophage increases inflammation of Type-2 Diabetes Mellitus in the liver. They found that TGR5-AKT-mTOR signaling pathway involves in obesity-induced insulin resistance by improving insulin action and modulating chemokine expression [34]. Interestingly, TGR5 expression suppresses inflammation and reduces atheroma plaques formation and thus decreasing the atherosclerosis effect in heart [35]. In bovine aortic endothelial cells, activated TGR5 induces nitric oxide productions and inhibits NF-κB activity in which suppresses monocyte adhesion and prevents the accumulation of atheroma plaques in arterial.

2.4 Muscarinic receptor

The muscarinic (M) receptors are classified into M1, M2, M3, M4, and M5. The muscarinic receptors are divided into two categories by which they inhibit adenylate cyclase (M2 and M4) or stimulate phosphoinositide hydrolysis (M1, M2 and M5). Historically, in brain, the selective effect of muscarinic receptor is suggested to be a therapeutic target for the treatment of Parkinson diseases by the modulation of muscarinic antagonist receptor [36]. The muscarinic receptor of eyes helps in protecting tear film and lens for the treatment against myopia among children [37]. In heart, muscarinic receptor stimulation by the parasympathetic nerves modulates contraction [38]. Cheng et al. reported that bile acid lithocholyltaurine is a ligand for M3 muscarinic receptor [39]. Binding of lithocholytaurine to M3 receptor of CHO cells stimulate acetylcholine-induced inositol phosphate formation and mitogen-activated (MAP) kinase phosphorylation [40]. Taurocholate (TC) shown to inhibit cAMP, affect calcium transient amplitude and reduce contraction of cardiomyocytes. Those effects were mediated by M2 muscarinic receptor [41]. Similarly, Ibrahim et al. [42] showed that conjugated bile acids action was mediated by M2 muscarinic receptor and not sphingosine 1 phosphate 2 receptor.

2.5 Sphingosine-1-Phosphate (S1P) Receptor

Bile acid has been reported to regulate Sphingosine 1 Phosphate (S1P) level. S1P is known to be the most potent substrate of sphingolipid. Activation or inhibition of S1P pathways can determine cell fate, whether it will undergo pro-apoptotic signaling or pro-survival signaling. There are 5 subtypes of S1P receptor, known as S1P1, S1P2, S1P3, S1P4 and S1P5 receptor. S1P receptors are GPCR; hence each receptor subtype downstream actions is determine by the G protein that couple to its receptor. In hepatocytes, the most abundantly expressed S1P receptor are S1P1 and S1P2 that are able to activate ERK 1/2 and AKT pathways by natural ligand for GPCR, S1P [43]. On the other hand, S1P1, S1P2 and S1P3 receptor are mostly abundant in heart whereas the expression of S1P4 and S1P5 are only limited to immune response and nervous system. S1P2 has been reported to be a receptor

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for conjugated bile acids, taurocholate (TCA) activating ERK 1/2 and AKT signaling pathways [44]. Studer et al. [44] show that inhibiting S1P2 expression with S1P2 antagonist, JTE-013 in primary rat hepatocytes significantly inhibits the hepatic ERK1/2 and AKT activation thus impeding the SphK2 production. Recently, S1P2 expression has become important in lipid metabolism by which they found that pertussin toxin (PTX) block ERK1/2 activation inhibits the S1P2 signaling which is suggested to be the upstream of epidermal growth factor receptor (EGFR)-mediated signaling in hepatocytes [45]. The concrete studies on the conjugated bile acids activated S1P2 are highly expressed in the mouse hepatocytes through real time reverse-transcription polymerase chain reaction (PCR) were done [46]. They found that mRNA level of the S1P2 expression was highly regulated in primary hepatocytes and in vivo. Interestingly, conjugated bile acid, taurocholate induced expression of S1P2 shows to promote cholangiocarcinoma growth [47]. In heart, endothelial cells abundant of S1P1 receptor which is required in the combination of Gi-mediated response to promote angiogenesis and other important cardiac cellular mechanisms [48].

2.6 Non-receptor mediated response

Studies have shown that apart from known bile acid receptor (FXR, LXR, VDR, PXR, TGR5, Muscarinic and S1P), BAs also activate non-receptor response that is the bile acid-sensitive ion channels, voltage- and Ca^{2+} - potassium (K+) (BK) channels. Bukiya et al. [49] reported that the litocholate (LC), a type of bile acids that activates BK channel through unique docking at ß1 subunit transmembrane domain 2 (TM2) bonding of the steroid ring enhances vascular myocytes BK channel activity [49]. High concentration of Bas is required for the activation of BK channels as compared to FXR or PXR. Bukiya et al. (2014) further listed the ligands that are possible as BK channels activator, leukotrienes and cholane steroids through lack docking of the hydrophobic reactions in LCA thus improving ion channels opening [50]. There are two possible actions of BAs to activate the ion channels, 1) direct binding of BAs to the channel protein by inducing its conformational changes for gate opening and 2) BAs interfere the plasma membrane of the cells by altering its lipid surrounding thus inducing sensitive channel opening [51]. The summary of BAs as signaling molecules is listed in Table 2.

Receptor	Tissue	Bile acid	Physiological implications	References
FXR	Liver Cholangiocytes Colonocytes Small intestine Heart	CDCA, DCA, LCA	Glucose metabolism, regulate cholesterol metabolism through repression of CYP7A1 resulting in less LDL-R expression and increase LDL-C levels in high bile acids pool concentration, triglyceride metabolism, bile acid metabolism by increase bile acid efflux, modulation of cellular proliferation and secretion, regulate bile acid homeostasis through transcriptional regulation of bile acids detoxification, intracellular binding and secretion, increased the digestion and absorption of ingested fats and anti-proliferative agent	[52-55]

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Others; PXR, LXR, VDR, S1P	Liver and Heart	LCA	regulate hepatic lipid metabolism, activate ERK 1/2 and AKT survival signaling pathway, regulation of lipid and glucose metabolism	[44, 56-58]
TGR5	Liver, Heart Dendritic cells,	TLCA, LCA, DCA, CDCA, CA, UDCA,	Modulate insulin signaling pathway via AKT activation by bile acids help to control hepatic glucose metabolism and inhibition of LPS-induced cytokine expression	[13, 59-60]
Muscarinic	Liver, Brain, Eyes, Heart and Colon carcinoma	Lithocholyltau -rine (LCT), Tauro-conjugate of cholic acid (TC)	Controls of hepatic glucose homeostasis, thermogenesis, energy homeostasis, inflammatory response, modulate muscarinic antagonist, protecting tear film and lens against myopia, stimulate parasympathetic nerves modulate contraction	[38,61]
Sphingosine-1-phosphate (S1P)	Cholangio-carcinoma, Heart, Liver	TCA	Promotes cholangiocarcinoma growth, lipid metabolism, promotes angiogenesis and cardiac cellular mechanism	[47-48]
Non-receptor-mediated BA actions; BK channel	Liver and intestinal tract	LCA	Enhances the ability of bile acids as a ligand and helps in bile acid transport and detoxification, and improves vascular muscle cells vasodilation.	[49-50,51]

Table 2: The main receptor involved in bile acids signaling

3. Bile Acid Signals in Heart

Taurocholate (TC) is a taurine conjugated hydrophobic BAs has been reported as a partial agonist of the muscarinic M2 receptor which induces arrhythmia in cultured CMs [41]. On the other hand, the most hydrophilic bile acid UDCA has been shown to protect cholestasis fetal heart model from BAs induced-arrhythmia [62-63]. Interestingly, Miragoli et al. [62] found that the cardioprotective effects of UDCA against BA-induced arrhythmia involving adenosine triphosphate potassium (K_{ATP}) channels and $[Ca^{2+}]_i$ level. In 1998, Bährle and co-workers reported a significant improvement of acute rejection episodes in heart transplant patients treated with UDCA compared to the untreated one [64]. However, the study by Bärhle is only done retrospectively. Therefore, the cardioprotection mechanism in heart transplant is not well understood. In ischaemia-reperfusion injury, Lee et al. [65] have shown that UDCA reduced lactate dehydrogenase release and enhanced

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the recovery of cardiac contractile function during reperfusion. In addition, UDCA showed protection effect for rat myocardium against reperfusion injury by inhibiting mitochondrial permeability transition pore (MPTP) dependent of PI3K/AKT pathway [66]. Rajesh et al. [66] further showed that UDCA protects in vivo and in vitro model of ischaemia-reperfusion injury by mediating the phosphorylation of Bcl-2-associated death (Bad) protein and prevents its translocation to mitochondria thus blocking the downregulation of Bcl-2 and the opening of MPTP. In patients with heart failure, the UDCA treatment was shown to improve endothelium and nitric oxide independent vasodilatation which maintains the arterial flow of impaired nitric oxide production [67]. In addition, another clinical study on patients with chronic heart failure was conducted, where the patients received 500mg UDCA twice daily for 4 weeks, and UDCA was observed to improve post-ischaemic peripheral blood flow in arms and legs. Apart from that, liver function was improved where the levels of γ-glutamyl transferase, aspartate transaminase, and soluble tumor necrosis factor α receptor 1 were lower after treatment with UDCA [68]. In 2013, a study reported UDCA as FXR agonist and led to the inhibition of NO synthase expression which would cause congestive heart failure and other CVDs [69]. Recently, Mahmoud and Elshazly [70] demonstrated the effect of UDCA in uric acid reduction and improvement of insulin resistant of fructose-induced metabolic syndrome rat which is associated with cardiovascular disease [70]. Studies have shown that UDCA upregulates survival signaling protein ERK 1/2 and Akt [71] and downregulates caspase-9 and ROS generation in CoCl2-induced hypoxic CMs [72].

4. Bile Acids Cause Abnormal Contraction and Calcium Dynamics

In isolated rat hepatocytes, bile acids cause an increase in cytosolic calcium level which is similar to the one seen in the presence of other agonists [73]. Several studies have proposed that taurine conjugates of LCA cause the release of intracellular calcium from inositol phosphate (IP3)-sensitive stores by permeabilisation of the endoplasmic reticulum [74]. In vascular endothelial cells, DCA, CDCA and taurine conjugates have been shown to increase in intracellular calcium mobilisation from IP3- sensitive stores. However, this is not seen in all cell types, for example it was not observed in human platelets or neuroblastoma cell lines (NG108-15) [75]. This effect of bile acids on calcium has therefore been suggested to be cell specific.

However, the effect of bile acid on intracellular calcium in cardiac cells is not well established. A previous study has demonstrated that a primary bile acid TC alters the rate and rhythm of CMs contraction and simultaneously causes abnormal Ca^{2+} dynamics in in-vitro model of neonatal rat CMs [76]. The addition of TC to neonatal rat cardiomyocyte cultures reduced the amplitude of contraction and caused dysrhythmias. Furthermore, the arrhythmogenic effect was different in individual cells and TC caused desynchronization of Ca^{2+} dynamics [77-76]. Recently, TC has been shown to cause the abnormal Ca^{2+} dynamics in the early but not late stages of mouse embryonic stem cell-derived CMs [41].

5. Hydrophilic bile acid

Around 1000 years ago, the traditional Chinese practitioner discovered that liver of the black bear could treat chronic liver diseases. At that time, the bear bile prescription was very expensive due to its least availability in the market. For the past 50 years, scientist has discovered that the liver of the black bear has high UDCA concentration. Recently, UDCA has been shown to reduce the cholesterol absorption and dissolve the cholesterol formed in gallbladder as an alternative medication apart of undergoes surgery. In addition, UDCA protects liver cells by reducing the elevated liver enzyme levels by facilitating bile flow through liver [79].

UDCA (Molecular formula = C24H40O4; Molecular weight = 392.572 g/mol) is synthesized in the intestine by bacterial epimerization of the CDCA at seventh carbon atom of the hydroxyl group (7-OH). CDCA and UDCA are different due to its hydroxyl group position in the steroid skeleton. UDCA hydroxyl group is positioned at β chair conformation cyclic compared to CDCA which is in α. The alpha isomer is less stable than the beta isomer. Therefore, UDCA is the most hydrophilic bile acid. It has the capability to permeabilize in cells easily, compared to other hydrophobic bile acid.

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Furthermore, UDCA has been shown to diminish the properties of hydrophobic bile acid-induced oxidative damage [80-82]. Commercially, synthetic UDCA is known as ursodiol, it is soluble in water and manufactured as a white or off-white crystalline powder with bitter taste.

6. UDCA as a Therapeutic Agent

UDCA is the tertiary bile acids and more effective compare to its taurine conjugated structure, TUDCA, in the treatment of inflammatory bowel disease (IBD) [83]. In liver and liver related diseases, UDCA is shown to improve biliary secretion in primary sclerosing cholangiocytes (PSC) and stimulates detoxification of hydrophobic bile acids in primary biliary cirrhosis (PBC). The up-regulation of efflux pumps expression such as MRP2, BRCP and P-glycoprotein in cells treated with UDCA shows that UDCA involves in stabilizing small intestinal detoxification [84]. In primary rat hepatocytes, UDCA is shown to down regulate p53 expression and prevent apoptosis signaling through reduction of Bax expression, mitochondrial translocation, and cytochrome c release [85]. Clinical studies show that UDCA intervention able to restore the liver function of obstructive jaundice patients after the endoscopic treatment [86]. Intrahepatic cholestasis (ICP) is a pregnancy liver disorder reported to affect pregnancy during the third trimester and mostly treated with UDCA. In ICP, the elevated serum bile acids could lead to maternal pruritus and increase of fetal bile acid constituent. UDCA is proven to decrease the toxicity effect of those elevated hydrophobic bile acids. Clinical studies performed have shown the experience of reduction of pruritus in pregnancy with UDCA treatment at 40% [87]. Moreover, studies show that UDCA relieves the effect of ICP stimulate hepatic intracellular secretion [88].

UDCA dissolves gallstone formation and prevents the related disease such as chronic cholecystitis, biliary colic, pancreatitis, or obstructive jaundice. UDCA is known as a non-surgically dissolution of gallstone recommended as a safe and effective agent compared to laparoscopic cholecystectomy operation. UDCA also tends to be more potent than chenodeoxycholic acid as a desaturating agent of gallstone dissolving of non-obese patients [89]. UDCA treatment on patients with cholesterol gallstone showed an improve contraction of gallbladder smooth muscle strips after six weeks of administration in compared to normal patients, thus suggesting that the administration of UDCA for longer periods increases the effectiveness of gallbladder contraction [90].

7. Mechanism of Action by UDCA

UDCA mechanisms of action could be divided into anti-apoptotic and pro-apoptotic signaling. In the hepatocytes, UDCA inhibits Fas-ligand-induced apoptosis in mouse hepatocytes postulated act through mitochondria membrane permeability [91]. UDCA effect on diverse hepatocytes disease shows that UDCA is a promising drug to treat other related liver diseases in future as it has shown to play a significance role in cholestasis, fibrosis, and sclerosis. Moreover, UDCA has shown to inhibit the effect of GCDCA-induced apoptosis which is independent of caspase -8/-3/-9 and dependent of anti-apoptotic kinase, for example p38, ERK 1/2 and PI3K [92]. Apart from liver, UDCA is also reported to have anti-apoptotic effect in other cells such as, cholangiocytes [93], brain [94], eyes [95] and osteosarcoma fibroblast. In contrast, same studies have shown that apart from anti-apoptotic signaling, UDCA play a roles in pro-apoptotic signaling in cancer cells such as gastric, prostate, and colon cancer cells.

UDCA activates a network of signaling pathway, which stimulates hepatobilliary exocytosis and insertion of transporter proteins into the membrane of the hepatocytes. Therefore, UDCA is known to overcome impairment of bile acid and toxic compound secretion from the hepatocytes canalicular membrane into the biliary tract. Those responses are mediated by protein kinase C alpha (PKCα) and $[Ca^{2+}]_i$. Moreover, in hepatocytes, UDCA can interact with membrane integrin and MAP Kinase [88]. During intrahepatic cholestasis pregnancy (ICP), hydrophobic bile acids accumulation leads to apoptosis and subsequently liver fibrosis [96]. Furthermore, UDCA protects placenta against BA-induced damage of isolated placenta in pregnancy of ICP and placental of ICP animals model [97]. Interestingly, report has shown that colon injury in alcoholic cirrhotic patients is due to high level of conjugated bile acids with increase of mRNA level of

pro-inflammatory cytokines compared to non-alcoholic drinker of cirrhotic patients who have high level of unconjugated UDCA [5]. A clinical trial conducted on cystic fibrosis patients reported that UDCA protects patients' liver and improves liver function test without affecting their lung function [98]. While in primary biliary cirrhosis (PBC) patients, UDCA treatment improves liver function without interfere their biochemical response at post-operational liver transplantation [99]. Murakami et al., [100] suggested the beneficial effect of UDCA as a potent drug to help in reducing blood glucose and stimulating glucagon-like protein 1 (GLP-1) in type 2 diabetes mellitus patients. Studies have suggested that UDCA might act as a ligand of GPCR receptor which leads to recruitment of phosphotidyl inositol-3 kinase (PI3K) protein and phosphoinositide dependent kinase 1 (PDK1). Activation of PI3K and PDK1 in rat hepatocytes reported to inhibit apoptosis [79]. Moreover, histopathological study shows that the inhibition of PI3K-Akt signaling increases the damage to the intestinal tissue of mice [101].

In cholesterol gallstone, UDCA reported beneficial in dissolving the stone formation. As UDCA is a therapeutic agent in cholesterol gallstone treatment, studies have suggested UDCA could be used to avoid many critical diseases such as atherosclerosis and cholesterol cholelithiasis. Recent treatment of gallstone by ezetimibe is reported less effective as UDCA, as ezetimibe only forms unsaturated micelle and inhibits cholesterol absorption through intestinal cells [102]. UDCA shows that it can completely dissolve gallstone by reducing intestinal absorption and biliary secretion of cholesterol [103].

UDCA anti-apoptotic effects have been reported in rat livers and isolated human hepatocytes [104]. In those studies, hydrophobic bile acids are reported to induce apoptosis in the hepatocytes. UDCA has shown to be cytoprotective against hydrophobic bile acids by inhibiting the formation of ROS and translocation of the pro-apoptotic protein Bax from cytosol to mitochondrial. While in Parkinson's in vitro model, UDCA can stabilize the mitochondria membrane by activation of the glucocorticoid receptor [105]. Apart from Parkinson's disease, the effect of TUDCA was reported in Alzheimer's patients by inhibiting the neuronal apoptotic death pathways [106]. The anti-apoptotic effect of TUDCA reported to inhibit amyloid β-peptide (Aβ)-induced apoptosis activating E2F-1 transcription factor and interfering in mitochondrial pathways that mediate p53 stabilization. Furthermore, overexpression of p53 in neuroblastoma cells treated with TUDCA was reported to reduce nuclear defragmentation and caspase activation such as the caspases 2 and 6 that are responsible in inducing the neuronal cell death [107]. In cultured mouse hypothalamic neuron, UDCA can increase the synchronicity of firing. Interestingly, the effect is inhibited in the presence of (GABA) receptor antagonist [108]. This study is in line with other studies that demonstrate UDCA activate glucocorticoid nuclear receptor and lead to the inhibition of apoptosis [109]. Therefore, UDCA is suggested to be a promising neuroprotective drug for neuronal-related diseases. Duan et al., [110] shows that nigral cells suspension pre-treated with TUDCA prior to transplant in the brain of rat Parkinson's disease model exhibited low apoptosis activity and increased the survival of grafted cells. Apart from that, UDCA effect on CMs and osteosarcoma fibroblast has also been reported [111]. UDCA shows to inhibit the effect of bilirubin and LCA induces apoptosis in osteoblastic cells by preventing translocation of pro-apoptotic protein BAX to mitochondria and decrease in caspase-3 activity.

UDCA has been suggested as an efficient and safe drug in treating retinitis pigmentosa, a progressive neurodegenerative disease of retina [112]. Fernández-Sánchez et al., [113] reported that systemic TUDCA treatment preserved retina structure as well as function of P23H-transgenic animal. Transgenic P23H albino rat are engineered to be a model of rhodopsin mutation which commonly occurs in human. This model develops a dysfunctional rod, and loss of photo receptor which leads to degenerative alteration in the inner retina. P23H rats treated with TUDCA demonstrated a significant reduction in TUNEL labelling which indicates that UDCA suppressed apoptosis. In cataract animal's model, UDCA and TUDCA have been shown to suppress choroidal neovascularation (CNV) formation and subsequently reduce the inflammation in retina [114]. Furthermore, TUDCA and UDCA treated cataract rats showed suppression of vascular endothelial growth factor (VEGF) in retina compare to untreated cataract rats. Apart from that, study on the cells

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and animals shows that UDCA prevents selenite-induced cataract in lenses by maintaining antioxidant status; GSH level, inhibiting peroxidation and decreasing MDA levels [115].

In cancer cells, hypoxia is shown to block Bax translocation to mitochondria by limiting the TRAIL-induced apoptosis and subsequently inhibit caspase-3 activation in human colon carcinoma (HCT116) cell [116] . Although UDCA has been reported by numerous studies as an anti-apoptotic agent, however in some cells and especially cancer cells, UDCA is reported as pro-apoptotic agent. Due to that, researchers mostly suggested UDCA effects are cell specific. In gastrointestinal cancer cell lines (SNU601) UDCA is reported to activate ERK 1/2 and caspases (-8,-3 and -6) and subsequently induced apoptosis [10]. UDCA is suggested as an anticancer agent which offers elimination of the apoptotic-sensitive and resistant cancer cells. Apart from that, UDCA shows to induce apoptosis in prostate cancer cells with the interaction of TGR5 receptor on the cell membrane [116]. In clinical trials, UDCA treatment reduced flutamide-induced hepatopathy incidence in prostate cancer patients [117].

8. Cardioprotection

Cardioprotection by definition includes all mechanisms and means that contribute to the preservation of the heart by reducing or preventing myocardial damage. It could lead to physiological adaptation or compensatory mechanism in reducing or preventing myocardial damage [118]. There is a vast range of cardioprotective drugs known to treat patient vulnerable to heart diseases. Cardioprotective drugs help to reduce the damage of the heart due to stress such as oxygen starvation. The most common prescribed drugs for cardioprotection are streptokinase [119], trimetazidine (TMZ) [120] and sulfaphenazole [121] which is used in the treatment of various heart diseases. Each drug plays a role in protecting the heart through a similar or different mechanism of protection. Heart disease may cause cardiac myocytes injury or loss. The CMs plays a major role in maintaining the physiology of the heart. Sufficient supply of oxygenated blood throughout the coronary arteries protects heart muscle and improves pumping ability of the heart. UDCA has shown to be important against heart diseases, as many studies had been done to increase the trust on consuming it as a therapeutic drug in future medicine. Recently, UDCA treatment is reported to improve peripheral blood flow in chronic heart failure patients [68]. During normoxia, p53 is a short-lived protein which is tightly regulated by the murine double minute-2 (Mdm-2) protein. Mdm-2 protein has been shown to inhibit p53 activity by binding to its transactivation domain and targeting it to ubiquitination. This leads to p53 translocation into the cytoplasm which promotes its degradation by the proteasome. Mohamed et al., [123] has shown that UDCA inhibits the upregulation of p53 and hif-1α in hypoxic cardiomyocytes. HIF-1 is used to target many genes such as p53, VEGF, nitric oxide synthase, PDK1 in mediating tumor metastasis, angiogenesis, energy metabolism and metabolic adaptation. In addition, HIF-1α is also involved in adapting low concentration of oxygen /survival and in apoptosis.

9. UDCA as Potential Drug for Cardioprotection

Ischemia is widely known as the main cause of acute myocardial infarction (AMI). AMI could be reduced and coronary blood flow could be restored using drugs such as streptokinase, tPA, antiplatelet agent and beta-blockers. Streptokinase is the most common drug used to treat myocardial infarction and it is normally given as therapy for less than 5 hours only. In addition, patients are also given reperfusion therapy. Reperfusion injury occurs due to oxygen restoration after ischemia and it has been treated using oxygen mask to supply enough oxygen to the tissue. A study shows that reperfusion could induce much greater injury in ischemic heart diseases patient [124]. However, streptokinase treatment could lead to side effect such as bleeding. Therefore, streptokinase treatment is suggested to be given between 30 to 60 minutes' interval to reduce the side effect. Apart from known streptokinase, trimetazidine is also a drug of choice for heart disease. TMZ protects heart by enhancing ATP levels of cardiac muscle and subsequently leads to the increase of coronary blood flow. Sulfaphenazole is another cardiac protective drug that inhibits

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cytochrome P450-induced myocardial ischemia-reperfusion injury [125]. As most of the drugs used for heart disease treatment and therapy could lead to unwanted effect, investigations of new cardioprotective agents are needed.

UDCA suggested being beneficial adjuvant in reducing cardiac allograft rejection of patients after heart transplantation [64]. Bährle et al, [64] reported reduction of acute rejection in patients treated with UDCA in comparison to patients of heart transplant without UDCA treatment. In another study, UDCA was reported in reducing lactate dehydrogenase (LDH) and improved contraction of ischemic rat heart model [65]. Furthermore, Lee et al., [65] reported that UDCA improved coronary flow in heart and post-reperfusion therapy model. Gorelik et al., [63] showed that UDCA protect heart from taurocholate-induced arrhythmias by improving contraction and calcium dynamic changes of ventricular rat CMs. In addition, this study suggested the cardioprotective effect of UDCA is similar to dexamethasone which alters the expression of genes for bile acid transporter and metabolism in CMs. Dexamethasone has been reported in protecting cardiac cells contraction rate during arrhythmia. Moreover, UDCA protects CMs against arrhythmogenic consequences of the more hydrophobic bile acid taurocholate (TC) which is mediated by the adenosine triphosphate-gated K+ (K_{ATP}) channels and $[Ca^{2+}]_i$ [62]. In 2012, 17 clinically stable patients of chronic heart failure were recruited for UDCA clinical trials [68]. From the clinical trials, they showed that 4 weeks of UDCA treatment significantly increased blood flow in arm and leg of post-ischemic patients. This reduction in leg and arm blood flow is known to be one of the factors affecting the life of patients with CHF. Treatment with angiotensin receptor blockers is known to improve cardiovascular illnesses in CHF, however the blood reduction in peripheral blood flow remain as a limitation in patients. Furthermore, the improvement of peripheral blood flow by UDCA in recruited patients is similar to patients of CHF with diuretic.

UDCA has shown to activate the survival signaling (AKT) of cardiac myocytes isolated from ischemia-reperfusion injury in animal model [66]. The in vivo studies by Rajesh et al., [66] showed that UDCA cardioprotection activates PI3K-AKT dependent pathway which inhibits phosphorylation of Bcl-2-associated death (bad) expression, downregulate Bcl-2 expression and subsequently decreases the pro-apoptosis signaling. In addition, the study showed that UDCA improved the myocardial ATP content and inhibited cytochrome c release to avoid apoptosis in ischemia-reperfusion. Apoptosis of CMs could be prevented by phosphorylated AKT which interacts with Bcl-2 associated X protein (BAX) and subsequently leads to reduction of pro-apoptotic signaling in CMs [126]. Activation of AKT signaling pathway promotes myocytes survival against ischemia-reperfusion injury [127]. Moreover, others reported that AKT signaling pathway plays a role in CMs survival pathway during intermediate and severe hypoxia [128-129]. AKT is the downstream effector of activated form PI3K subunit shows to play a role in myocytes contractility [130]. Recently, the protective effect of UDCA on human fetal heart fibroblast and neonatal rat CMs are shown to be very much similar [131]. The study showed that UDCA treatment increases hyperpolarization in both human fetal heart fibroblast n neonatal rat CMs during chronic hypoxia. In addition, [71] reported that UDCA leads to upregulation of ERK and AKT in protecting cardiomyocytes from hypoxic condition.

Neutral SMase activation is detected in post-MI CHF patients and inhibition of nSMase activity leads to failure of recovery in the hearts of post-infarcted patients [132]. In heart, abundance of sphingomyelin in the cells promotes atherogenesis and might lead to angiographic coronary heart disease (CHD) and left ventricular systolic dysfunction [133]. In addition, secretory aSMase shown to be upregulated in CHF patients and the upregulation of this enzyme is reported to be associated with loss of functional capacity in skeletal muscle [134]. Furthermore, CHF patients are recorded to express 90% more of total SMase compared to healthy patients. Additionally, the study found that high total SMase in CHF leads to functional and structural impairment of skeletal muscle tissue. Empinado et al., (2014) reported that in CHF rat models there are high accumulation of nSMase, ceramide and S1P but there is no changes in aSMase and sphingosine activity [135]. Moreover, nSMase activation was reported to be highly elevated in hypoxia-reoxygenated CMs [136]. S1P1 receptor which is abundantly expressed in the heart, promotes angiogenesis and other important

cardiac cellular mechanisms [48]. Activation of S1P1 receptor known to affect cardiac contractility, heart rate, induce hypertrophy, provides protection from ischemia and mobilized $[Ca^{2+}]_i$. Clinical studies show that total SMase induces ceramide formation which leads to plaque rupture of atherosclerotic coronary artery disease (CAD) [137]. Ceramide accumulation induced myocardial infarction in post-ischemic heart of aSMase knockout mice [138]. SMases was suggested as important target in cells protection by regulating ceramide accumulation in the cells [139]. UDCA is shown to inhibit colon carcinoma development by activation of sphingomyelinase [140]. In unpolarized colon cancer, UDCA is reported in inducing alkSMase and subsequently inhibits cells proliferation [141]. In addition, UDCA treatment leads to reduction of aSMase and nSMase in colon cancer cells. The study demonstrated that UDCA exert its anti-proliferative effects in unpolarized colon cancer cells by regulating alkSMase, aSMase and nSMase levels. The polarized cells resemble the matured absorptive enterocytes on the microvilli, short lived and destined to apoptosis but the unpolarized cells that undergo differentiation and proliferation. In polarized colon cancer cells, UDCA shows to increase the alkSMase expression without affecting other types of SMases, and caspase 3 activity increases as the cells proliferation rates are decreased. In another study, UDCA was reported to inhibit axomethane-induced colonic tumor growth [142]. The study shows that tumor development was inhibited with UDCA alone and UDCA supplemented with carcinogen diet. S1P is a secreted ligand that activates growth factors, GPCR agonist and cytokines. It is important in protecting the plasma membrane of the sphingolipid bilayer which helps to regulate cell adhesion; to promote migration, differentiation and survival [143]. In addition, secreted S1P acts as a second messenger that binds to GPCR-S1P receptor to initiate cell proliferation and survival. There are 5 different types of S1P receptor; S1P1, S1P2, S1P3, S1P4 and S1P5. In rat hepatocytes, conjugated bile acids has shown to promote cytoprotective effect through S1P1 activation, ERK and AKT signaling pathways [44]. In heart, S1P1 is abundantly expressed. S1P receptors regulate cardiac physiology and pathophysiology. In addition, S1P shows to inhibit aSMase induced cell death in bone-marrow derived macrophages by prevent ceramide accumulation [144]. S1P1 receptor is found exclusively bound to Gi sensitive protein coupled receptor [48]. S1P1 expression activates the inhibition of cAMP formation and antagonizes adrenergic receptor-mediated contractility. While, S1P2 receptors activity in cardiac myocytes is mediated cardioprotection by activation of Rho. In addition, S1P3 receptors are also expressed in heart and its activation results in bradycardia [145].

10. Conclusions and Perspective

Bile acid signaling via receptor dependent (FXR, LXR, VDR, PXR, TGR5, S1P, Muscarinic) and independent (BK channel) mechanisms is widely studied on different types of cells. The ability of BAs as signaling molecules in liver diseases such cirrhosis and interahepatic cholestasis of pregnancy are well known. In this paper, our review focuses on bile acids as a signaling molecule or ligand to activate cellular metabolisms in most of the tissues. Furthermore, this paper provides an insight of BAs as ligands in protecting cells against the development of cardiovascular diseases.

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