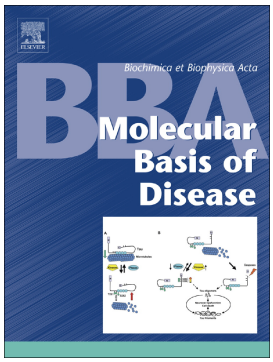

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Heart and bile acids – clinical consequences of altered bile acid metabolism

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Key words: bile acids, cirrhotic cardiomyopathy, intrahepatic cholestasis of pregnancy, primary biliary cholangitis, primary sclerosing cholangitis, ursodeoxycholic acid

Abstract

Cardiac dysfunction has an increased prevalence in diseases complicated by liver cirrhosis such as primary biliary cholangitis and primary sclerosing cholangitis. This observation has led to research into the association between abnormalities in bile acid metabolism and cardiac pathology. Approximately 50% of liver cirrhosis cases develop cirrhotic cardiomyopathy. Bile acids are directly implicated in this, causing QT interval prolongation, cardiac hypertrophy, cardiomyocyte apoptosis and abnormal haemodynamics of the heart. Elevated maternal serum bile acids in intrahepatic cholestasis of pregnancy, a disorder which causes an impaired feto-maternal bile acid gradient, have been associated with fatal fetal arrhythmias. The hydrophobicity of individual bile acids in the serum bile acid pool is of relevance, with relatively lipophilic bile acids having a more harmful effect on the heart. Ursodeoxycholic acid can reverse or protect against these detrimental cardiac effects of elevated bile acids.

1.1 Bile acid synthesis

Bile acids (BAs) are synthesised in the liver via cholesterol catabolism in a multi-enzymatic pathway; the rate- limiting step being the initial conversion by the cytochrome P450 enzyme CYP7A1. The primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA) are subsequently conjugated in the liver with amino acids taurine or glycine. Subsequent to secretion into the gut during food digestion, intestinal microbiota cause their deconjugation and dehydroxylation via 7-alpha-dehydroxylase, forming the secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA) respectively [1]. The majority of BAs are reabsorbed in the intestine and return to the liver for re-uptake via the portal vein, a process known as the enterohepatic circulation. BAs characteristically have an amphipathic nature and their hydrophobicity is linked to their conjugation with amino acids. Conjugated BAs are more hydrophilic, and their resulting decreased membrane permeability causes a reduction in cytotoxic potential [2].

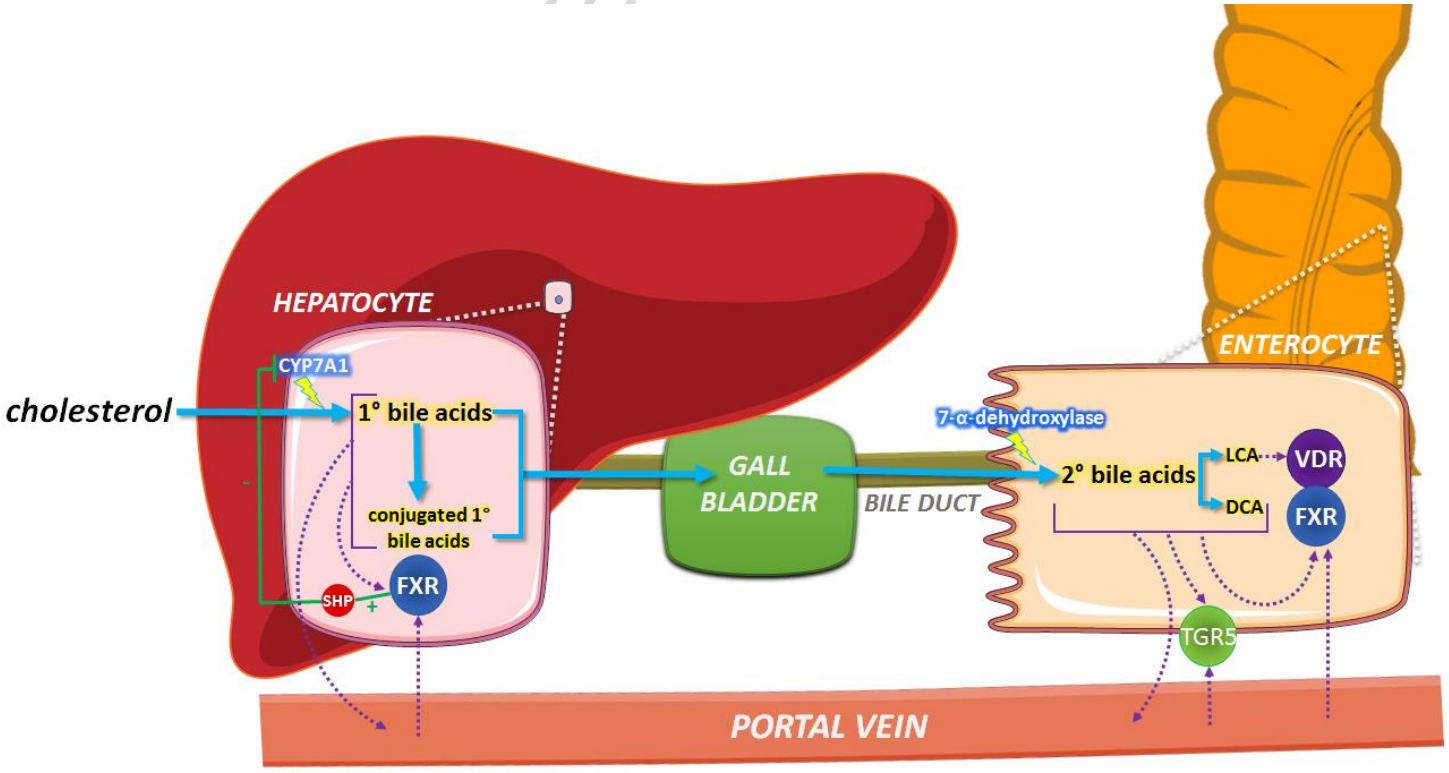

Figure 1: Bile acid (BA) synthesis and signalling: Primary BAs are synthesised and conjugated in the liver and are deconjugated and dehydroxylated in the intestine to form secondary BAs. Their signalling occurs through various pathways, and highlighted here is the nucleus-based activation of Farnesoid X receptor (FXR and the enterocyte-expressed Takeda G protein-coupled receptor (TGR5) by primary and secondary BA ligands as well as the nucleus-based activation of vitamin D receptor (VDR) by LCA.

1.2 Electrical conduction and the mechanics of the heart

Normal cardiac function requires the co-ordination of electrical activity and mechanical contraction of the ventricles and atria of the heart. This is initiated via the spontaneous generation of an action potential by the sino-atrial node, propagating to the atrioventricular node via the right and left atria, causing the atria to contract, and then for blood to fill the ventricles that are in their relaxed or diastolic phase. Ventricular depolarisation occurs, and once ventricular pressure surpasses atrial pressure, the atrioventricular valves close, causing a state of isovolumetric contraction in the ventricles. The continuation of this signal through the distal fibres of the atrioventricular node (known as the bundle of His) subsequently cause the right and left ventricles to contract, and this electrical signal is then propagated via the Purkinje fibres to cardiomyocytes, allowing the ejection of blood by the ventricles, known as ventricular systole. A state of isovolumetric relaxation then occurs, forming the end of one cardiac cycle and reintroduction into the diastolic phase of the ventricles. The cardiac cycle can be observed using electrocardiography (ECG), where the QRS complex which results from recording electrical activity represents the stages of ventricular depolarisation. The PR interval refers to the time taken from atrial depolarisation to ventricular depolarisation and

the QT interval refers to the time taken for ventricular depolarisation and repolarisation to take place. Abnormalities in the length of either interval can be indicative of atrial or ventricular tachyarrhythmias respectively [3, 4].

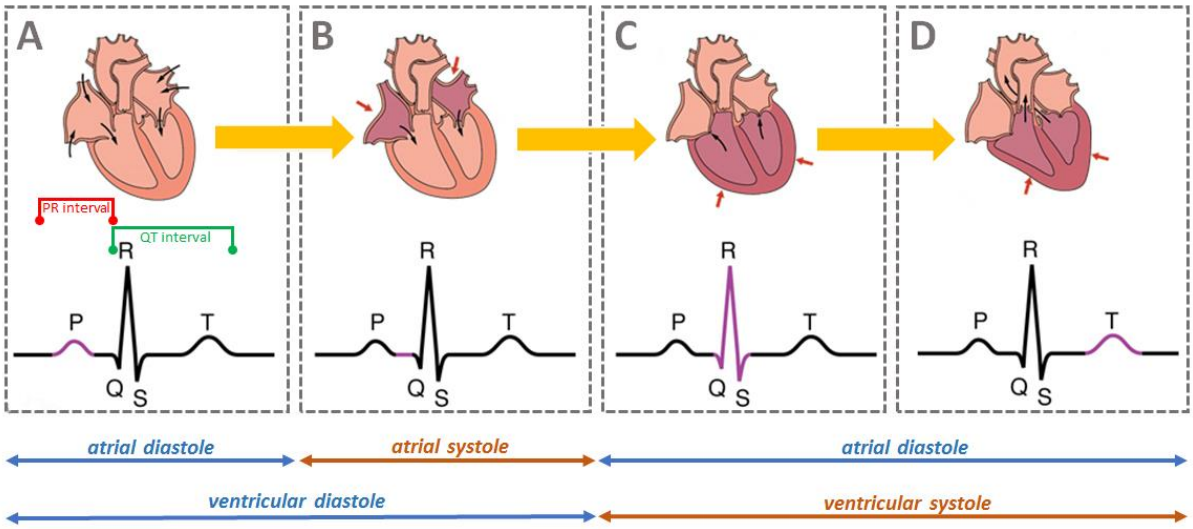

Figure 2: The blood flow through the heart during the cardiac cycle in correspondence with the QRS complex observed in electrocardiography: (A) Atrial filling during atrial diastole (B) Atrial systole and ventricular filling (C) Ventricular isovolumetric contraction (D) Ventricular systole and ejection of blood from the ventricles. The measurement of the PR and QT interval values using the QRS complex is also indicated in (A).

1.3 Bile acid receptors are expressed in the heart

It is now known that receptors which mediate BA signalling are also expressed in cardiovascular tissue. The nuclear BA receptor farnesoid-X receptor (FXR), whose main ligands are the primary BAs has been shown to be expressed in the vasculature [5]. Activation of vasculature-specific FXR improves lipid profiles and influences vascular

tension, thereby resulting in an anti-atherosclerotic effect [5, 6]. Low levels of FXR expression were detected in neonatal cardiomyocytes; however no functional response of these receptors was induced [7]. Recent work by Pu et al has also demonstrated the presence of FXR in adult cardiomyocytes and cardiac tissue, and activation of cardiomyocyte FXR via *in vitro* CDCA administration significantly induced FXR mRNA expression. *In vitro* administration of CDCA also resulted in a dose and time-dependent apoptotic response in these cells. Opening of the Mitochondrial Permeability Transition Pore (MPTP), a protein involved in apoptotic signalling and associated with heart failure, was also found to be induced by FXR activation [8, 9]. Potentially clinically relevant data show that FXR expression was significantly upregulated in ischaemic cardiac tissue in rats and inhibition of FXR reduced the size of insult, suggesting that FXR plays an important role in mediating cardiac apoptosis and injury [8].

Expression of the vitamin D receptor (VDR) has been localised in the t-tubules of cardiomyocytes [10]. In addition to vitamin D, the secondary bile acid LCA is known to be a ligand for this receptor. Cardiomyocytes isolated from $vdr^{-/-}$ mice have increased rates of contraction and activation of the receptor in wild type mice also resulted in altered contractility when vitamin D was used as a ligand rather than LCA [10]. Vitamin D exposure also altered proliferation and morphology of cardiomyocytes [11]. Selective deletion of the VDR in cardiomyocytes resulted in myocyte enlargement, cardiac hypertrophy, systolic and diastolic dysfunction [12]. There is therefore evidence of VDR expression and functional activity of relevance to normal cardiomyocyte function. However, whether these pathways are also influenced by LCA-liganded VDR has not been demonstrated.

The expression of Takeda G protein-coupled receptor 5 (TGR5), also known as G protein-coupled bile acid receptor 1 GPBAR1, mRNA has been identified in moderate levels in human, rabbit and bovine heart tissue, as well as mouse tissue and cardiomyocytes at a protein level [13-15]. Although TGR5 expression in the heart has been demonstrated, its function remains unknown, however, certain studies have shown that TGR5 does respond to BA administration. Mouse cardiomyocyte-specific TGR5 responded to administration of taurochenodeoxycholic acid (TCDCA) and LCA by downregulating glycogen synthase kinase-3β (GSK3β) and upregulating protein kinase B (AKT), which are known to be associated with cardiac hypertrophy [15]. In addition, TGR5 expressed in the endothelium of the bovine aorta produced Nitric Oxide (NO) in a dose-dependent manner in response to taurolithocholic acid (TLCA) administration [16].

Experiments on neonatal cardiomyocytes demonstrated the ability of taurocholate (TCA) to mediate muscarinic M2 receptor-induced alterations in contractility [7] (see later).

1.4 Cholestatic liver disorders are associated with impaired cardiovascular function

Total serum bile acid (TSBA) concentrations are known to increase in patients with liver dysfunction [17]. In general, it has been observed that patients with liver disorders have cardiac and haemodynamic changes with increased cardiac output and decreased vascular resistance [18]. The cardiotoxicity of BAs was observed as early as 1863 with the discovery that bile acid administration induced bradycardia and eventually cause cardiac arrest [19]. The relationship between BAs, liver disorders and cardiac dysfunction was initially investigated in detail in 1953 by Kowalski et al, who observed that patients with liver

cirrhosis had a prolonged QT interval [20]. Further observations of cirrhotic patients coupled with the knowledge that BAs are significantly elevated during liver dysfunction gave rise to the suggestion that BAs are one of the components in the mechanism of cardiac dysfunction.

The two most characterised cholestatic liver disorders are Primary Biliary Cholangitis (PBC) (also known as primary biliary cirrhosis) and Primary Sclerosing Cholangitis (PSC), both of which have been associated with poor cardiovascular function. PBC, a disorder more commonly observed in women, results from the lymphocytic inflammation and later destruction of intrahepatic bile ducts, leading to cirrhosis and an increase in TSBA concentrations [21]. Similarly, PSC, a disorder more commonly seen in men, results in the inflammation of the biliary epithelium of the intrahepatic and extrahepatic bile ducts [22]. Currently, the primary FDA approved drug for treatment of both these disorders is ursodeoxycholic acid (UDCA). UDCA is a relatively hydrophilic BA which is thought to displace more hydrophobic BAs in the BA pool and therefore shift the composition to a less toxic one; the importance of which will be discussed later in this review [22-24].

1.4.1 Primary Biliary Cholangitis and Primary Sclerosis Cholangitis increase the risk of cardiovascular disease

There is a known association between patients with cholestasis and an impairment in cardiovascular function, and several population studies have sought to highlight these associations and define the alterations in risk.

The relative risk (RR) of cardiovascular disease after onset of PSC is 3.34, but this is primarily arterial or venous disease [25]. PSC has also been demonstrated to cause dyslipidaemia and changes in low density lipoprotein (LDL) cholesterol; however, lipid profiles do not appear to correlate with risk of cardiovascular events. This may be attributed to the high concentration of lipoprotein-X (LP-X) found in PSC patients, an abnormal LDL that has been shown to have anti-atherosclerotic effects. However, the small sample size in the study suggests a lack of conclusive data to establish the role of dyslipidaemia and further investigation is required [26]. A single case study has shown that a patient who has been diagnosed with PSC and epilepsy had impaired ventricular function, specifically ventricular tachycardia with a short coupling interval, although the aetiology for this disorder was unknown [27].

In contrast to PSC, there are more data regarding the link between PBC and cardiovascular dysfunction. In the most recent study, the RR of cardiac events in comparison to controls was found to be 2.2 [23]. Patients with PBC have dyslipidaemia with increased levels of serum cholesterol, primarily LP-X, that decrease in later stages of the disease. However this again is not associated with an increased risk of cardiovascular events, a result which again may be attributed to small sample size or the anti-atherosclerotic effects of LP-X [28].

Earlier studies showed that patients with PBC have significant prolongation of the corrected QT (QTc) interval [29]. Impedance cardiography has shown that PBC patients have an abnormal left ventricular (LV) ejection time in response to tilting from a supine to upright position. Interestingly, this response was not seen in PSC patients [30]. Magnetic Resonance Spectroscopy also indicated significant reduction of markers of cardiac muscle energy

function [30]. Studies have also shown that PBC results in a significantly reduced heart rate variability (HRV) and baroreflex sensitivity, both of which were associated with the incidence of fatigue, a commonly observed symptom in patients with PBC [31]. Patients with early-stage PBC had reduced thoracic fluid content which in turn affected myocardial contractility and diastolic function; this was independently associated with markers of cardiac inotropy or contractility [32]. A case report also described right ventricular dysfunction in one patient 7 years after diagnosis with PBC [33].

Alagille syndrome, an autosomal dominant disorder caused by a mutated JAG1 or NOTCH2 gene, results in a cholestatic state due to the lack of interlobular ducts in the liver. Patients with this syndrome commonly present with cardiac murmur and are also diagnosed with Tetralogy of Fallot (TOF), the most common form of cyanotic congenital heart disease [34]. TSBA concentrations in patients with TOF have also been correlated with right ventricular function [35].

Biliary atresia is a rare disorder that presents in infancy, however is the most common cause of liver transplants and cirrhosis in that age group [36]. Infants with biliary atresia awaiting liver transplantation have been shown to exhibit pathologies in cardiac structure and function including significant increases in LV and septal wall thickness and LV shortening fraction. 72% of infants in one study displayed features of cirrhotic cardiomyopathy prior to transplantation, a syndrome which is described in more detail in the next section [37].

1.5 Cirrhosis can result in the development of Cirrhotic Cardiomyopathy

Cholestatic disorders can eventually result in cirrhosis of the liver [38]. It is estimated that approximately half of all cases of liver cirrhosis result in the development of cirrhotic cardiomyopathy (CC), a disorder characterised by systolic and diastolic dysfunction, morphological changes and abnormalities in the electrophysiology of the heart [39].

CC is caused in part by electrophysiological abnormalities; including the inability to respond to certain pharmacological stimuli, the disruption of the contraction response of cardiomyocytes to electrical excitation and a prolonged QT interval. The latter symptom in particular delays ventricular repolarisation and lays the groundwork for a possible ventricular arrhythmia to occur. A prolonged QT interval is associated with increased risk of sudden death and mortality due to this potential to cause arrhythmias [40].

A morphological abnormality that has been observed with different degrees of severity in cirrhotic patients of one study is left ventricular hypertrophy, which has been shown to eventually lead to diastolic dysfunction [41]. The most common electrophysiological abnormality seen in patients with CC is an increase in QT interval length compared to non-cirrhotic controls [40]

It is thought that numerous factors are involved in the development of CC, however there is evidence that BAs play an important role. The relationship between BA metabolism and cardiac dysfunction or CC has been determined with both *in vitro* systems and experimental models in intact animals. Direct effects of BA acid exposure can be observed *in vitro* using

isolated cardiomyocytes and muscle strips. The indirect effect of BA metabolism can also be determined through animal models of cirrhosis which are known to result in CC. Bile duct ligation (BDL) is the primary method to produce such a model but other models of cirrhosis can be generated by other methods such as portal vein stenosis, carbon tetrachloride (CCl4) or 3,5-diethoxycarbonyl-1,4-dihydroxychollidine (DDC) feeding [15, 42]. Important data on the effects of impaired BA metabolism on the heart has also been identified in humans with cholestatic liver disorders as described above for PBC and PSC.

1.5.1 Bile duct ligation allows mechanisms of Cirrhotic Cardiomyopathy to be uncovered

The pathogenesis of CC is multi-factorial and complex, however manually obstructing the bile duct itself has allowed experimental investigation into some of the underlying mechanisms involved. BDL, mainly in the rat and mouse, results in rodent models of what we now know as CC, and has therefore allowed the investigation into the relationship between BA metabolism and the associated cardiovascular phenotype.

Early experiments observed that BDL of rats causes a decrease in heart rate and significant biochemical changes in the serum, with increased bilirubin peaking at 5 days post ligation. ECG analysis has also shown prolongation of PR and QT intervals [43, 44]. Aside from the rat model, CC via BDL has also been shown in dogs, which also display features characteristic of CC such as increased cardiac output and electrophysiological abnormalities [45].

The importance of apoptotic pathways in contributing to CC was identified by Nam et al, whose studies of cardiomyocytes isolated from BDL rats showed a significant increase in apoptotic markers e.g. poly ADP-ribose polymerase (PARP) and also an increase in systolic and diastolic function upon administration of anti-FasL antibody, an inhibitor to the apoptotic Fas pathway. This effect was not observed in cardiomyocytes isolated from their sham counterparts. This evidence suggests that myocardial apoptosis can be induced by BDL and will in turn lead to CC [46]. Further investigation has identified the role of NO in cardiomyocyte apoptosis. Hearts harvested from BDL mice have increased rates of apoptosis and morphological abnormalities and inhibition of NO synthase in these mice resulted in improvement of both of these parameters, suggesting that it acts as the mediator [47]. These results are in agreement with studies which show administration of increased concentrations of DCA and CDCA on isolated vascular endothelial cells *in vitro* results in the increase of NO production via intracellular Ca^{2+} signalling, investigated using whole cell patch clamping [48]. Increased apoptosis was also observed in BDL mice using a terminal transferase deoxyuridine triphosphate nick end labelling assay (TUNEL), and this was significantly decreased with the addition of the endogenous opioid naltrexone, suggesting that blocking of opioid receptors can modulate apoptosis, however this did not appear to affect LV diastolic function [49].

BDL appears to induce significant haemodynamic changes in the rat including increased cardiac output, hypotension and basal bradycardia. These changes were reversed upon administration of cholestyramine, an intestinal BA sequestrant, suggesting that BAs have a direct role in the pathogenesis of these parameters [50]. The hormone erythropoietin has also been shown to have a cardioprotective effect on BDL rats, reversing systolic and diastolic dysfunction and lowering oxidative stress via the decrease of expression of contractile

inhibitory factor TNFα [51]. BDL-induced cirrhosis in rats also has an inhibitory effect on cardiac mitochondria, reducing capacity for oxygen consumption and ATP synthesis, [52, 53].

Ma et al have demonstrated that BDL rats have decreased cardiac membrane fluidity which results in beta adrenergic dysfunction and inability to produce cAMP, resulting in blunted contractile ability [54, 55]. The expression of titin and collagen (regulators of passive tension in cardiac muscle) are altered in BDL rats, suggesting that abnormal expression of these proteins contribute to contractile dysfunction in CC [56]. Isolated monocytes from BDL rats have been shown to reduce cardiomyocyte contractility *in vitro* [57, 58]. Inducing haemorrhage in BDL rats significantly reduced LV contractility, a change that was quickly reversed in controls. This was associated with the release of endocannabinoids, a known component of the stress-response system in the heart, suggesting a mechanism of why CC causes impaired cardiovascular response [57]. Previous studies have shown that cytokines such as interleukin-1beta (IL-1β) mediate cardiomyocyte contractility via nitric oxide synthase-2 (NOS2) in BDL-induced cirrhosis [59].

Murine models of BDL-induced CC have therefore highlighted several different mechanisms by which cirrhosis causes cardiovascular complications. The explicit effect of BA metabolism on cardiac function can also be observed in murine models via *in vitro* and *in vivo* experiments where direct BA exposure has been performed.

1.5.2 Exposure of the heart to relatively hydrophobic bile acids results in cardiac dysfunction

BAs can be directly administered in murine models to expose their direct role in cardiac function. In these experiments, it is important to consider the specific species of BAs, as relative hydrophobicity plays a role in determining their effect.

Early *in vitro* experiments with cardiomyocytes provided the initial impetus for determining the effect of bile acids on the heart. Incubation of sera from jaundiced rats on cultured cardiomyocytes caused their contractions to slow down and eventually cease; an effect also seen with DCA treatment in isolation. This gave rise to the hypothesis that DCA was the component in jaundiced sera causing the cardiomyocyte phenotype [60].

In addition to the BDL experiments described above, Joubert et al also found that injection of CA resulted in dose-dependent negative chronotropic effects. Bradycardia was observed in most rats at doses of >10mg/kg, an effect that was slightly reduced by removal of the vagal nerve and increased when administered in the vein rather than the artery, giving rise to the suggestion that BAs cause both direct and vagally-mediated hypotensive effects [43].

Binah et al demonstrated that *in vitro* administration of primary and secondary BAs at concentrations as low as 10nmol/L on rat ventricular muscle resulted in negative inotropic effects, including a reduction in active tension as well as the rate of tension activation and relaxation. This corresponded with a reduction in the duration of the ventricular action potential. Incubation with sodium taurocholate resulted in changes in the inward calcium

(Ca^{2+}) and outward potassium (K^{+}) currents that generate the contraction action potential, suggesting that BAs affect membrane potential and hence induce alterations in inotropism [61].

The effect of sodium taurocholate on the sino-atrial node *in vitro* has been studied previously in the rabbit. Physiological-mimicking concentrations of >30μmol/L had effects on the action potential of the sino-atrial node, decreasing the time to peak. Higher concentrations of >100μmol/L decreased the rate of diastolic depolarisation and resulted in prolonged bradycardia. Both inward Ca^{2+} and outward K^{+} currents of the sino-atrial node were also slowed, in agreement with the earlier results seen by Binah et al [62].

Recent detailed studies in murine models have further linked elevated BAs and CC and investigated the underlying mechanisms. Inducing biliary fibrosis, cholestasis and cholanaemia in mice via DDC feeding resulted in elevated markers of cardiac dysfunction, including the decrease of heart rate and cardiac mass and increase in ejection fraction, all of which correlated with the increase in circulating BA levels. In addition, expression of genes which regulate fatty acid oxidation (FAO) and are induced in stress response were correlated with BA concentration. CA feeding in bile salt export pump (BSEP) deficient mice also demonstrated this effect, confirming a role for BAs as important mediators of cardiac dysfunction. Reversal of liver injury using a normal chow diet showed a recovery in biochemical, structural and electrocardiographic abnormalities [63].

More recently the *in vivo* and *in vitro* effects of BA administration in *Fxr* and *Shp*-deficient mice have been studied. Double knock out (DKO) mice (lacking both these genes required

for BA homeostasis) exhibited cardiac hypertrophy, reduced cardiac output, bradycardia and prolonged QTc and PR intervals together with elevated markers of myocardial remodelling and injury; thus validating this strain as another model of CC. Injection of 100mg/kg of TCA and LCA mimicked these effects in wild type mice, as did acute administration of these BAs via perfusion. *In vitro* CDCA treatment of cardiomyocytes (isolated from wild type mice) at pathological concentrations led to metabolic dysfunction and the suppression of expression of fatty oxidation regulatory and glucose oxidation genes, a term they coined "cholecardia", a pathology which could explain the contractile disturbances in diseases associated with elevated serum BA levels. This effect was also seen in vivo and ex vivo via DKO mice and perfusion of DKO mouse hearts respectively. Reducing the BA pool in DKO mice via cholestyramine resulted in the restoration of cardiac function, including heart rate, cardiac output and systolic and diastolic function [64].

Further evidence of the effect of BAs on cardiac mitochondria has been shown by Ferreira et al, who isolated cardiac mitochondria from wild type rats and exposed them to various physiological concentrations of BAs *in vitro*. Exposure to relatively more hydrophobic BAs (LCA, DCA and CDCA) resulted in alterations in mitochondrial energetics, including a decrease in the mitochondrial respiratory ratio and membrane potential, resulting in MPTP activation. The relatively hydrophilic BAs glycochenodeoxycholic acid (GCDCA), taurodeoxycholic acid (TDCA) and glycoursodeoxycholic acid (GUDCA) had less of an effect. GUDCA was the most hydrophilic BA tested and had the lowest mitochondrial toxicity index [53].

Zavecz and Battarbee compared the effect of CA in mouse ventricular muscle strips *in vitro* to the *in vivo* cirrhosis effect induced by portal vein stenosis (PVS) in rats. They found that administration of CA caused similar reductions in contractile tension as were seen in PVS rats at concentrations as low as 1nmol/L. Administration of UDCA did not increase this tension in combination with CA; however chronic gavage of UDCA in PVS rats partially reversed the decrease in contractile tension. This suggests that the phenotype reversal upon UDCA treatment was due to displacement of hydrophobic BAs in the BA pool of the PVS rats rather than direct exposure [42]. CA exposure also resulted in decreased beta-adrenergic response, decreased cAMP production, reduced concentration of intracellular stores of calcium and reduced sodium-calcium exchange activity, all of which are associated with cardiomyocyte contraction [42].

It has also been shown that TCA, GCA and GCDCA cause a dose-dependent induction of arrhythmic contractions in adult human atrial trabeculae, with TCA in particular causing a significant induction in arrhythmias at concentrations of ≥30μmol/L *in vitro*; corresponding with the TSBA concentrations seen in patients with cholestasis [65]. UDCA appeared to reverse this atrial fibrillation, corresponding with the attenuating effect seen in the experiments described above. Rainer et al also showed via logistic regression analysis of 250 patients who had heart failure that hydrophobic BA concentrations act as predictor of atrial fibrillation [65].

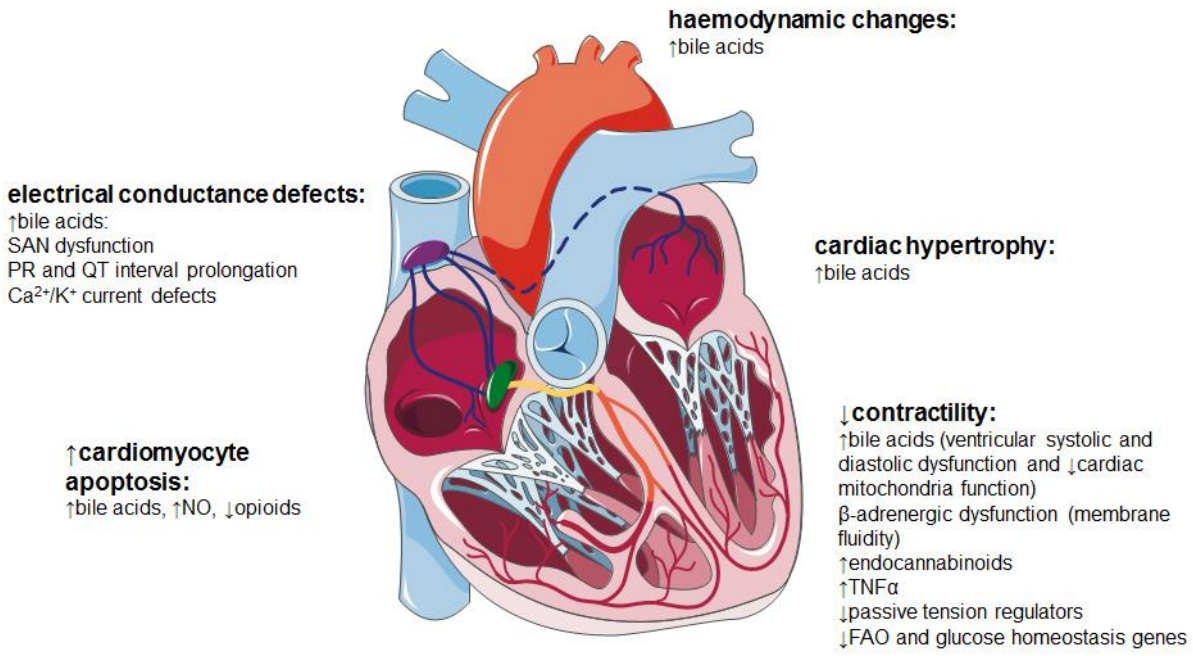

Figure 3: The pathogenesis of cirrhotic cardiomyopathy (CC): Development of CC is complex and multifactorial, however defects in the regulation of bile acid metabolism have been demonstrated to heavily influence the development of CC characteristics.

The above studies illustrate the direct effect of BA exposure on cardiac function, as well as demonstrating the link between the magnitude of these effects and the hydrophobicity of the BAs involved. Desai et al showed that CC can be reversed in mouse models upon normalisation of BA concentrations and recovery of DDC-induced cirrhosis using normal chow feeding. UDCA appears to have a protective effect on cardiomyocytes and atrial trabeculae *in vitro*, and the effect on neonatal cardiomyocytes will be discussed later in this review. The effect of UDCA on adult cardiac function *in vivo* has also been documented, as described below:

1.5.3 UDCA has a protective effect in adult cardiac disease

The proven efficacy and safety of UDCA in hepato-biliary disease has made it an increasingly popular drug, and this has promoted exploration of the potential benefit of its use in other disease areas, including adult cardiac conditions such as myocardial infarction and atherosclerosis.

Sudden cardiac death (SCD), caused by acute myocardial infarction (AMI), is a frequent cause of death in developed countries [66-69]. Typically caused by atherosclerosis and thromboembolic events; occlusion of a coronary artery results in complete or partial ischemia of the downstream myocardial tissue, followed by the development of an infarction. It is well known that restoration of the blood flow to previously ischemic myocardium results in a range of reperfusion-associated pathologies named 'ischemia-reperfusion (IR) injury'. This has a considerable impact on health and society, not only due to the acute-phase mortality caused, but also because of the long-time morbidity and mortality [70]. Based on the protective effects of UDCA observed in liver IR injury, effects on the heart have been studied and, interestingly, UDCA has been found to prevent IR injury and cardiac infarction. Pre-clinical studies have been conducted by Lee et al. in 1999 and Rajesh et al. in 2005 [71, 72]. In the isolated heart perfusion model, Lee et al. showed that UDCA (80-160μ) reduces IR damage following 30 minutes of global ischemia. The beneficial effects included improved left-ventricular diastolic pressure (LVDP), enhanced contractile function and reduced release of lactate dehydrogenase (LDH), a well-known marker of cellular integrity [71]. Later, Rajesh et al. evaluated the effects of UDCA pre-treatment on ischemia-reperfusion in an anaesthetized rat model. Animals were treated either with UDCA (40mg/kg) or vehicle for 30

minutes prior to left-coronary artery occlusion for 180 minutes, followed by a 180 minute period of reperfusion. From histological analysis it emerged that animals treated with UDCA had a reduced infarcted area following reperfusion. This was proposed to be mainly due to the capacity of UDCA to inhibit the mitochondrial permeability transition pore via activation of the phosphatidylinositol 3 (PI3) kinase pathway [72].

Tauro-conjugated UDCA, TUDCA (the main species seen in humans), administered to rats prior to a myocardial infarction (MI) also exhibits anti-apoptotic effects and improves cardiac function. In a recent study TUDCA (50 mg/ml, 400 mg/kg, IV) or PBS was administered to rats and then the left anterior descending (LAD) coronary artery was ligated. Animals were sacrificed 24 hours later and a significant reduction in apoptotic cells was found in the rats pre-treated with TUDCA. Caspase-3 activity, an early apoptotic marker, in the TUDCA treated animals also decreased. In addition, transthoracic ultrasound examination of heart function was performed at 1 and 4 weeks post-ligation. By 4 weeks, a significantly smaller infarct area was present in the TUDCA group compared to the PBS group. There was also an improvement in shortening fraction (SF) in the TUDCA-treated animals. Therefore TUDCA may be considered as a viable treatment for reducing apoptosis in a model of myocardial infarction in rats [73].

The same authors previously showed in a rodent model of acute stroke that TUDCA decreases brain infarct size by nearly 50% when compared to the controls [74]. This makes TUDCA a plausible drug for stroke treatment. TUDCA was given in a single dose (400 mg/kg, IV) during or 1 hour after temporary ischemia-reperfusion. Two days later, the rats were euthanized and their brains were sectioned and stained with 2% 2,3,5-

triphenyltetrazolium chloride (TTC). Quantitative analysis of the infarct size showed a significant reduction of infarct volume (49.0% in the vehicle group vs. 24.2% in the treatment group, $p < 0.05$). Furthermore, TUDCA reduced the number of TUNEL cells as compared to controls, indicating less DNA fragmentation [74].

TUDCA has been identified as a chemical chaperone, modulating endoplasmic reticulum (ER) stress and therefore acting to attenuate cardiomyopathy. A recent study by Rani et al found that oral administration of TUDCA in a mouse model of transverse aortic constriction resulted in reduction of ER stress markers and cardiac hypertrophy. A reduction in cardiac apoptosis was also seen in this model after chronic administration, as well as an apparent reduction in cardiac remodelling including myocardial fibrosis, collagen deposition and TGF-beta signalling [75]. TUDCA has also been shown to reverse high fat-induced alterations in cardiomyocyte contraction, mitochondrial permeation pore opening and phosphorylation of insulin signalling molecules [76]. This is in agreement with the effect of UDCA observed in a genetic model of obesity, the $ob^{-/-}$ knockout mouse [77]. TUDCA has also been shown to attenuate angiotensin II induced abdominal aortic aneurism in apolipoprotein E deficient mice [78].

Cholesterol and lipoproteins (low-density lipoprotein (LDL) and high-density-lipoprotein (HDL)) play an important role in the development of plaques and atherosclerosis lesions. Since bile acid synthesis is the key route of cholesterol elimination in the body, increasing bile acid production by UDCA may play an important role in the prevention of atherosclerosis. Potential benefits have been shown for UDCA in the reduction of LDL and increase in HDL in both pre-clinical and clinical studies [79-81]. Coupled with the

cholesterol lowering and anti-inflammatory effects of UDCA, the proposed capacity of UDCA to act as endogenous vasodilator makes it an attractive treatment for congestive heart failure. Patients suffering from PBC receiving UDCA (13mg/Kg/day) over a month, showed a reduced diastolic volume without any systolic, diastolic or mean blood pressure change [82]. In a separate study in coronary artery disease patients, 6 week therapy with UDCA (13-19mg/Kg) improved endothelium-dependent nitric oxide-independent vasodilatation [83].

The effects of UDCA on endothelial function and inflammatory markers was assessed in a prospective, single-centre, double-blind, randomised, placebo-controlled crossover study in clinically stable male patients with CHF (chronic heart failure) (New York Heart Association functional class II/III, LV ejection fraction <45%) [84]. Patients received in random order 500 mg UDCA twice daily for 4 weeks and placebo for another 4 weeks. The primary endpoint was post-ischemic peak peripheral arm blood flow as assessed by strain-gauge plethysmography. UDCA was well tolerated in all 16 patients that took part in the trial. Compared with placebo, UDCA improved peak post-ischemic blood flow in the arm (+18%, $p = 0.038$), and a trend for improved peak post-ischemic blood flow in the leg was found (+17%, $p = 0.079$). At the same time levels of γ-glutamyl transferase, aspartate transaminase, and soluble tumour necrosis factor-α receptor 1 were lower after treatment with UDCA than after placebo (all $p < 0.05$). This shows significant improvement of the liver function in these patients. However, there was no change in 6-min walk test or New York Heart Association functional class, and levels of TNFα and interleukin-6 were unchanged or increased compared with placebo. The study concluded that UDCA improves peripheral blood flow and liver function in patients with CHF.

Recently, UDCA has been assessed for protecting effect against immune mediated organ transplant rejection. The capacity of UDCA to prolong graft survival and increase the amount of transplant tolerance observed in rat models has been reviewed from clinical studies with a significantly lower incidence in acute rejection episodes in the UDCA (500mg) group, compared to control [85, 86].

Taken together, evidence from pre-clinical and clinical studies show that, UDCA may be a novel effective therapeutic strategy in different cardiac conditions as a consequence of its protective effect against cell damage

1.6 Human studies investigating the relationship between bile acids, liver disorders and cirrhotic cardiomyopathy

A prolonged QT interval is the most commonly observed feature in CC. The exact mechanism by which CC increases the length of the QT interval is unknown, but there is mounting evidence that a multitude of factors play important roles. A study by Bernardi et al found that serum concentrations of CA and CDCA were significantly positively associated with prolongation of corrected QT (QTc) interval length suggesting that serum BAs are involved in this systolic defect. However serum BAs alone are not enough to act as an independent predictor of QTc prolongation with respect to the severity of liver disease [87].

Child-Pugh scores, which are used to grade the severity of liver cirrhosis, have been shown to be positively associated with the likelihood of a prolonged QTc interval [88]. Child-Pugh

scores themselves in hepatitis-B induced cirrhosis have been significantly correlated with serum levels of GCA, GCDCA, TCA, TCDCA and GUDCA, and there is a potential for serum BAs to act as biomarkers for severity of cirrhosis [89].

Bal et al showed that 40% of their cohort of 409 patients with chronic liver disease had a prolonged QTc interval, which occurred more commonly in patients who had alcohol-related cirrhosis as well as correlating with the age and Child-Pugh score [90]. A study by Genovesi et al has also shown that the type of cirrhosis is concordant with the extent of QTc interval prolongation, as the incidence was greater in patients with alcohol induced cirrhosis in comparison to viral cirrhosis [91]. Differences in alcohol and non-alcohol cirrhosis is contradictory to Bernardi et al's study in which no significant difference was observed [87]. In addition to Bernardi et al, other studies have shown that the type of cirrhosis does not indicate the likelihood of QT interval dysfunction [92].

The results of an echocardiogram study by Cichoz-Lach et al observed prolongation of the QTc interval, but this did not correlate with the Child-Pugh score [93]. However, cirrhosis was also found to significantly reduce QRS voltage, which did correlate with Child-Pugh classification [93].

1.6.1 Liver transplantation treats prolonged QT interval, however TIPS worsens it

Liver transplantation in patients with CC appears to dramatically improve cardiac parameters, for example reduction of ventricular wall thickness, diastolic and systolic function as well as

exercise response [94]. The prolonged QTc interval in Bal et al's cohort was normalised in 55% of patients after liver transplantation and a similar normalisation effect was seen on a study of children with chronic liver disease and patients with end stage liver disease post-orthotopic liver transplantation [95-97]. In one study, the QTc interval was shortened in 87% of cases after liver transplantation [90].

Interestingly, this coincides with results where the gender-specific differences seen when examining QT intervals in normal patients (whereby healthy women have longer baseline QTc intervals than healthy men) have been investigated. The differences appear to be abolished in cirrhotic men and women and are not restored after liver transplantation, suggesting that deficiency of androgens does not contribute to cirrhosis-induced QTc interval prolongation, although QTc interval prolongation again did not appear to correlate with the severity of cirrhosis [98]. This is in contrast to previous suggestions that sex hormone metabolism does indeed play a role [99].

Transjugular intrahepatic porto-systemic shunt (TIPS) insertion, a treatment to relieve portal hypertension and the accumulation of bile salts in systemic circulation (caused by portal vein stenosis described earlier in Zavecz and Battarbee's experiment) was thought to be a likely intervention to improve prolonged QTc interval. However one study where TIPS was performed on cirrhotic patients with portal hypertension surprisingly had a negative effect and resulted in the further prolongation of QTc interval at both early (1-3 months post TIPS) and late (6-9 months post TIPS) observational time-points in the study, suggesting that portal hypertension and the resulting accumulation of cardioactive substances does not contribute to a prolonged QTc interval [100]. This study has a relatively small number of patients whose

prolonged QTc interval did not correlate with Child-Pugh score or other cirrhotic clinical parameters as seen in other studies. However the findings correlate with other investigations of QTc interval after TIPS [101].

1.7 Diagnosis of Intrahepatic Cholestasis of Pregnancy is determined by serum bile acid concentrations

Normal pregnancy is known to cause a slight elevation of maternal TSBA concentration. Intrahepatic Cholestasis of Pregnancy (ICP) is a disorder where the maternal TSBA concentration is elevated above the normal range and results in the accumulation of BAs in fetal serum causing an impairment in the transplacental gradient of BAs [102].

Elevated maternal TSBA concentrations of above 10µmol/L in combination with maternal pruritus in absence of a rash is usually considered diagnostic for ICP [103]. ICP is the most common pregnancy-specific liver disorder and is associated with the adverse fetal outcomes of fetal hypoxia, meconium staining, preterm birth and intrauterine death. ICP is commonly treated with UDCA and maternal TSBA concentrations of above 40µmol/L are sometimes described as severe ICP and are associated with an increased risk of adverse fetal outcomes [103, 104].

Investigation of women with mild and severe ICP compared to gestation-matched controls has shown a significant positive correlation between the concentration of fasting TSBA

concentrations and QTc interval dispersion, therefore indicating TSBA concentrations appear to be associated with the incidence of ventricular arrhythmia [105].

Umbilical cord blood collected from ICP patients has significantly elevated BA concentrations, and there is a significant reduction in the BA concentration of maternal and cord blood after treatment with UDCA. Whilst maternal BA profiles returned to a state similar to control women, UDCA had no significant effect on the composition of the fetal BA pool, although it did appear to increase the concentration of unconjugated BAs [102].

The RR of intrauterine death in ICP in comparison to uncomplicated pregnancy is 2.58, and it has been hypothesised that this risk is associated with fetal cardiac dysfunction or arrhythmia stemming from the increased fetal BA caused by the maternal cholestatic state [104, 106]. Therefore, the unexplained occurrence of intrauterine death could therefore be due to a BA-associated sudden cardiac event which has been described in several case reports summarised below.

1.7.1 Case reports of fetal arrhythmia in Intrahepatic Cholestasis of Pregnancy

It has been reported that ICP causes arrhythmias in the fetus, with UDCA treatment resulting in mixed outcomes. Al Inizi et al reported a patient who has been diagnosed with ICP at 37 weeks of gestation, experiencing fetal tachyarrhythmia followed by atrial flutter after induction of labour. The cardiac dysfunction ceased after the safe delivery of the fetus [107]. In a report by Shand et al, an individual with ICP developed fetal supraventricular

tachycardia (SVT) at 28 weeks of gestation. SVT is the most common form of fetal tachyarrythmia which may be associated with an increased mortality risk, especially in association with hydrops fetalis [108]. The patient was treated with anti-arrhythmic drugs together with UDCA and safe delivery of the fetus was achieved [109]. This is similar to a recent report by Altug et al, whereby an ICP patient also presented with fetal SVT. Treatment was with UDCA and anti-arrhythmic drugs, and SVT was only resolved after the induced delivery of the fetus [110]. One study of a pregnant patient who was previously diagnosed with PSC demonstrated that high levels of circulating TSBA concentrations in the fetus appears to correlate with fetal compromise, including the occurrence of fetal bradycardia [111]. Lee et al have reported two specific cases where raised serum BAs were observed and diagnoses of ICP were made. Although UDCA resulted in the decrease of TSBA concentrations in one case, both cases results in prolonged fetal bradycardia after onset of labour and ultimately observed the sudden demise of both fetuses regardless of constant fetal heart rate monitoring by cardiotocography (CTG) [112]. A previous case report of a patient diagnosed with ICP whose TSBA concentration was reduced by UDCA described sudden fetal demise prior to the onset of labour despite normal BA profiles and CTG monitoring [113].

1.7.2 Investigations into fetal ventricular function in Intrahepatic Cholestasis of Pregnancy

Doppler and Fetal ECG have been used to further investigate the fetal arrhythmia in ICP. Elongation of the PR interval has been reported in fetuses of patients with ICP [106]. PR interval elongation in fetuses is a detrimental abnormality which has been demonstrated to

cause arrhythmias and increase mortality risk [114, 115]. A pilot study by Strehlow et al demonstrated that ICP was positively associated with a significantly longer fetal mechanical PR interval, specifically by 14ms. Most of the ICP mothers were being treated with UDCA and had a mean TSBA concentration of 28.3µmol/L compared to 6.2 µmol/L concentration seen in the control group [116]. Though this study had a small sample number, similar findings have been observed in Rodriguez et al's larger cohort, in which fetal PR intervals from patients with ICP was also significantly longer, in this case by 13ms [117].

In addition to effects on the PR interval, other measures of ventricular dysfunction have been investigated in fetuses of patients with ICP. Ataalla et al found that fetuses of patients with severe ICP and TSBA concentrations of >40µmol/L had higher diastolic myocardial velocity as measured by tissue Doppler imaging of the right and left ventricular and septal walls [118]. LV global longitudinal strain, (a measure of LV function), systolic and diastolic strain rate was found to be significantly reduced in fetuses of patients with severe ICP who had TSBA concentrations of >40µmol/L. The strain rate was also correlated with levels of circulating N-terminal prohormone of brain natriuretic peptide (NT-proBNP) in fetal cord blood, a protein that is elevated in response to left ventricular dysfunction [119]. Fetal myocardial performance index (MPI) also known as Tei Index is a measure systolic and diastolic function and was also analysed in patients with ICP. The mean left MPI in the fetus was significantly positively correlated with TSBA concentration and isometric volume relaxation time was prolonged in patients with severe ICP, a measure which can been seen as an indicator of diastolic dysfunction [120].

In vitro experimentation using BA administration on isolated neonatal cardiomyocytes has provided supporting evidence of the above clinical data and probed the effect of UDCA on the fetal heart.

1.7.3 Induction of cholestasis in neonatal models of the heart and the protective effect of UDCA

Primary culture of neonatal cardiomyocytes has mostly been used as a model to study the effect of BAs in the heart. This is the closest available *in vitro* model of fetal myocardium as these cells beat spontaneously (even in a single cell culture) and synchronously in clusters/monolayer [121]. TCA (0.1 – 1.0mmol/L) reduces the rate and amplitude of contraction, of both individual neonatal cardiomyocytes and clusters; at a higher dose (3.0 mmol/L) cells completely stop beating [106]. The effect of TCA can be attributed to the direct effect of TCA on cardiomyocyte membrane depolarisation and alteration of calcium dynamics [122]. It is thought to be mediated, at least in part, via acetylcholine muscarinic M_2 receptor activation [7].

During development the fetal heart undergoes a 'physiological hypoxic' state which is required for neovascularisation as well as development of other systems [123]. This hypoxic period leads to the conversion of fetal cardiac fibroblasts into myofibroblasts. Recently this conversion has been confirmed to happen in human fetal ventricular tissue during the second and the third trimester of gestation, the period when cholestasis-related sudden fetal death is most commonly occurring [124].

A more complex and realistic *in vitro* model of the fetal heart has been developed, consisting of a co-culture of neonatal cardiomyocytes and myofibroblasts; this model has been compared to sole myocyte culture which acts as a maternal heart model [116].

Both acute and chronic treatment with TCA has been shown to reduce impulse propagation in the fetal heart model, but not in the maternal heart model. This may explain the higher vulnerability of the fetal heart to the exposure to high BA concentrations in ICP. In addition, acute treatment of TCA at 0.5 mmol/L causes early after-depolarisations, and results in sustained re-entrant arrhythmias (Figure 4). Interestingly, co-treatment with UDCA protects against the arrhythmogenic effects of TCA in the fetal heart model. These results also suggest that the protective effect of UDCA is more potent when myofibroblasts are present. In this study, UDCA, but not TCA or other BAs, produces a substantial hyperpolarisation of myofibroblasts in the fetal heart model. This may be due to an increase in potassium conductance, as direct binding of UDCA has previously been shown to the sulfonylurea receptors (SURs) expressed in cardiac myofibroblasts [125]. Moreover, UDCA associates with several inwardly rectifying potassium ion channels (K_{ir}) and KATP subunits [126, 127].

In the fetal heart model, TCA has been shown to depolarise neonatal rat cardiac myofibroblasts but not cardiomyocytes [128]. Similar effects of TCA were also observed in human fetal cardiac fibroblasts in culture [128]. Additionally, TCA prolongs calcium transient duration in the rat fetal heart model and human fetal cardiomyocyte culture. In contrast UDCA has been shown to hyperpolarise both rat and human fibroblasts, and abolish

the effect of TCA on the resting membrane potential [128]. Furthermore, treatment with UDCA normalises the effects of TCA on calcium propagation [128].

Interestingly, UDCA was also found to suppress the transition of neonatal rat cardiac fibroblasts and human fetal cardiac fibroblasts into myofibroblasts in hypoxic condition, as seen by the expression of alpha-smooth muscle actin (α-SMA), and thus preventing fibrosis, as shown by repression of several fibrosis markers [128]. This suggests that UDCA may be useful in the treatment of fibrosis. However, further studies are needed to investigate the underlying mechanisms of UDCA-induced suppression of the transition of fibroblasts to myofibroblasts.

With regard to the adverse effect of ICP on the fetal heart, most experiments have focused on the effects of TCA in neonatal heart models, even though elevation of other species of BAs (e.g., conjugated CDCA and DCA) occur in ICP (although not as markedly as TCA) [129]. Other BAs may negatively affect the function of the fetal heart in ICP pregnancies through different pathways than those described for TCA.

Desai et al. (2017) has reported high BA levels switching the metabolic function of cardiac cells from lipid oxidation to glucose metabolism. Treatment of neonatal rat cardiomyocytes with CDCA (100μmol/L) for 4 hours induces remarkable suppression of FAO regulators such as *Pgc1α, m-Cpt1*, *Nrf-1, Nrf-2,* and *Tfam*, as well as *Pdk4*, a crucial inhibitor of glucose oxidation. These finding suggest other mechanisms of BA action in modulating cardiac functions [64].

The involvement of other BA receptor signalling such as pregnane-X-receptor (PXR), VDR, TGR5, and sphingosine 1-phosphate receptor (S1P) has not yet been determined either in short or long-term BA exposure towards the fetal heart during ICP.

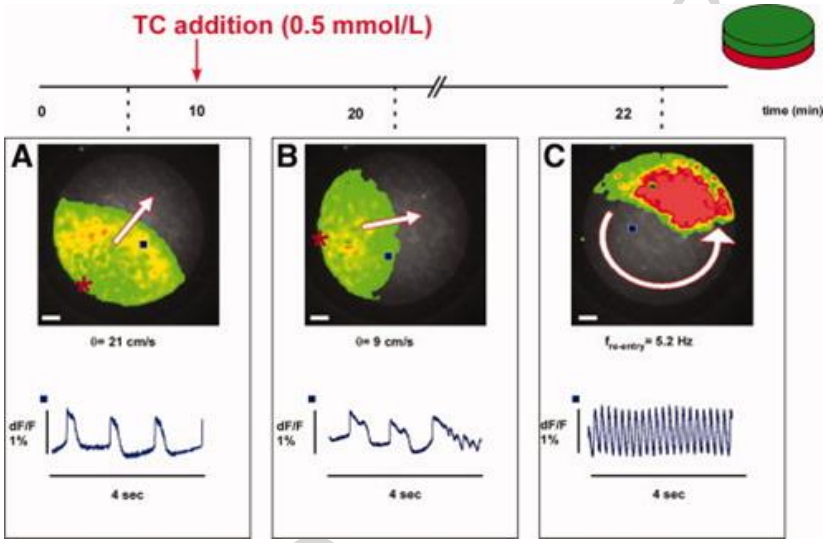

Figure 4: Example of the arrhythmia induced by taurocholic acid (TCA) in a preparation consisting of a monolayer of cardiomyocytes coated with a monolayer of myofibroblasts. (A) Optical recording of spontaneous electrical activity (45 bpm; bottom) originates from the periphery and propagates uniformly at 21 cm/s. (B) After acute exposure to 0.5 mmol/L, TCA conduction velocity (θ) was reduced from 21 to 9 cm/s. Moreover, propagated action potentials display EADs, where the last activation was followed by (C) self-sustained re-entrant excitation. Frequency of rotation was 5.2 Hz. Bar = 1 mm. Blue squares in the overview indicate the locations of recorded traces. Red stars indicate the origins of spontaneous electrical activation.

1.8 Summary and Future Perspectives

Previously it was assumed that the principal role of bile acids was to influence cholesterol catabolism and act as detergents for lipid absorption. However, increasing evidence has emerged for the role of BAs as hormone-like signalling molecules in a variety of physiological processes, one of these being normal cardiac function.

Expression of BA receptors on cardiomyocytes suggests that circulating BAs have the potential to directly affect the heart, although the functional ability of these receptors to mediate BA signalling has only been confirmed to date with the FXR and M_2 receptor pathways in *in vitro* cardiomyocyte cultures.

Cirrhosis results in impaired BA transport and is known to cause the development of cirrhotic cardiomyopathy in 50% of patients. Experiments to elucidate the mechanisms of how cirrhosis, BAs and cardiovascular function are related have been conducted on bile duct ligated rats, mice and other animal models of cirrhosis. Clinical observations of patients with cirrhotic cardiomyopathy together with patients with cholestatic diseases such as PBC and PSC have identified associations between TSBA concentration and severity of cardiac dysfunction. Specific analyses of the electrical conduction in the cardiac cycle have identified elongation of the QT interval in adults and elongation of the PR interval in fetuses to be associated with elevated TSBA concentrations.

In vitro and *in vivo* studies have established the clear contrast between the cardiotoxic effect of hydrophobic BAs and cardioprotective effect of hydrophilic BAs, thereby highlighting the importance of BA pool composition in cholestasis. The investigation of the mechanisms of action of UDCA has provided further evidence of the importance of BA pool composition due to the displacement of the more hydrophobic BAs upon UDCA treatment.

UDCA has been shown to improve cardiac function via a reduction in cardiomyocyte apoptosis and is often used as a treatment for liver disorders. TUDCA has also been shown to have a preventative effect on cardiac dysfunction in murine models. Ongoing clinical trials investigating Obeticholic acid (OCA), a derivative of CDCA and a more potent ligand for FXR, have also demonstrated considerable potential to reduce markers of liver cirrhosis in patients with PBC and PSC however it's specific effect on cardiac function has not been investigated [130]. Results from current trials using 24-norursodeoxycholic acid (norUDCA), a side chain shortened homologue of UDCA, have also displayed promise to treat PSC due to its ability to reduce cholestatic markers [131]. It is possible that hydrophilic BAs such as UDCA, UDCA derivatives and OCA have the potential to be used as stand-alone treatment for cardiac dysfunction, although the direct effect of these bile acids on the adult and fetal heart requires further investigation.

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Highlights

- Studies show that elevated serum bile acids are associated with cardiac dysfunction
- Bile acids have been implicated in the formation of cirrhotic cardiomyopathy
- Bile acids with a higher hydrophobicity have an increased dysfunctional effect