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Bilirubin Safeguards Cardiorenal and Metabolic Diseases: a Protective Role in Health

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Abstract

Purpose of Review—To discuss recent advances indicating that bilirubin safeguards against cardiorenal and metabolic diseases.

Recent Findings—Several investigations from human patient populations and experimental animal models have shown that bilirubin improves cardiorenal and metabolic dysfunction. The latest studies found an entirely new function of bilirubin suggesting that it acts as a hormone signaling molecule capable of activating nuclear receptors for burning fat, which may explain several of its protective actions.

Summary—This review highlights the current findings (within the last 3 years) regarding cardiorenal and metabolic protective effects of bilirubin and the latest mechanism(s) that may be mediating these effects.

Keywords

Biliverdin reductase; PPARalpha; Kidney; Hypertension; Metabolic syndrome

Introduction

Bilirubin is derived from the catabolism of heme by heme oxygenase (HO), which releases biliverdin, carbon monoxide (CO), and iron [1, 2]. Bilirubin (unconjugated) is formed by the reduction of biliverdin to bilirubin via the biliverdin reductase (BVR) enzyme [1], which is supported by recent studies in global BVR deficient mice that completely lacked serum bilirubin [3]. Plasma levels of bilirubin are thought to be derived from the breakdown of red

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blood cells in the spleen; however, more recent studies have shown that other tissues also have high expression of BVR [4]. Bilirubin is released into the blood where it binds albumin and travels to other organs or is deposited in the liver and conjugated with glucuronic acid by the UDP-glucuronosyltransferase 1A1 (UGT1A1) enzyme [5, 6]. Conjugated bilirubin (CBR) is excreted from the liver to bile in the gallbladder and eventually released into the intestine where it is further broken down to urobilinoids by the gut microbiota and eliminated in the feces [5].

Extraordinarily high levels of serum-unconjugated bilirubin (UCB) are responsible for jaundice (> 150 μM), which is a pathological condition resulting in the yellowing of the skin and eyes. Jaundice in adults is usually a symptom of severe liver disease. However, neonatal jaundice may be from other causes and can result in a condition known as kernicterus, which can cause brain damage at extremely high bilirubin levels (> 300 μM). Despite its role in jaundice, mildly elevated UCB (15–50 μM) has several beneficial effects on the body [7]. The results of numerous large-scale population and epidemiological studies have correlated a protective effect of increased serum bilirubin levels against cardiovascular and metabolic disorders [8••, 9•]. Bilirubin treatment was also shown to be beneficial in several animal models of cardiovascular and metabolic disease [10, 11]. However, the molecular mechanism by which this occurs is not fully understood. The objective of this review is to highlight recently published studies on the effects of bilirubin on the cardiorenal and metabolic systems and delve into the potential mechanism(s) of bilirubin's actions.

Bilirubin and Cardiorenal Protection

The health benefits of bilirubin in the cardiovascular system have been mainly revealed by human population studies, which have demonstrated a negative relationship between serum bilirubin levels and the development of cardiovascular disease [12•, 13]. The protective effects of mildly elevated serum bilirubin levels have been shown in patients with Gilbert's UGT1A1 polymorphism, which reduce UGT1A1 in the liver, increasing plasma bilirubin levels [14•]. Many different factors contribute to cardiovascular disease, including hypertension, atherosclerosis, inflammation, and overproduction of reactive oxygen species (ROS). Bilirubin intervenes in several pathways known to mediate the development and progression of many of these cardiovascular diseases (Fig. 1). For example, bilirubin interferes with vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) to regulate atherosclerotic lesion formation and vascular inflammation [15, 16]. The beneficial effect of bilirubin to attenuate atherosclerosis in animal models is consistent with the findings in humans, demonstrating the relationship between serum bilirubin levels and atherosclerosis in at-risk patients [17]. Mildly elevated circulating levels of unconjugated bilirubin negatively impact platelet activation by reducing P-selecting expression and also attenuate platelet aggregation to arachidonic acid [18]. Thus, bilirubin plays a multi-faceted role in the prevention of atherosclerotic disease.

Recent investigations have found a role of the immune system in hypertension [19–21]. Bilirubin modulates the immune response at several levels including modulation of regulatory T cells, modulation of T helper type 17 (Th17) cells, inactivation of the Nlrp3 inflammasome, and inhibition of the Toll-like receptor 4/nuclear factor kappaB signaling

pathway [22–25]. Not only can bilirubin modulate the immune system, but also, the BVR enzyme plays a vital role in immune function [26]. Biliverdin can inhibit the expression of complement activation fragment 5a receptor one (C5aR1) and deletion of the BVRA isoform from myeloid cells results in an increase in C5aR1 expression and enhanced chemotaxis towards C5a [27, 28]. In addition, BVR plays a regulatory function in macrophage polarization heavily favoring the expression of the anti-inflammatory cytokine IL-10, which is a marker for anti-inflammatory M2 macrophages [29]. BVR aids in the generation of M2 macrophages following ischemia-induced acute kidney injury and could be a possible therapeutic target for kidney disease [29].

In the heart, higher serum bilirubin levels are associated with improvements in left ventricular remodeling. Inoue et al. showed that low plasma bilirubin levels (< 0.8 mg/dl) in asymptomatic patients with type 2 diabetes mellitus was associated with a higher prevalence of concentric left ventricular remodeling compared to those with higher (≥ 0.8 mg/dl) levels of plasma bilirubin [30]. Additionally, elevated serum bilirubin levels are associated with improvements in heart disease [31]. These cardioprotective results are consistent with those derived from animal studies. Investigations in the hyperbilirubinemic Gunn rat have demonstrated that these rats exhibit reduced cardiac contractility, lower rates of aortic pressure development, significant aortic dilation, and increased aortic diameter [32]. In addition, Gunn rats are protected from cardiac ischemia-reperfusion injury [11]. It should be noted that the serum levels of bilirubin in the Gunn rat are incredibly high (~ 30 mg/dL or 500 μM) due to complete loss of hepatic UGT1A1 activity in this strain [33]. Studies utilizing an animal model with a more physiological increase in serum bilirubin similar to what is observed in humans with Gilbert's polymorphisms (~25–50 μM) are necessary to test whether moderate increases in serum bilirubin have similar protective actions against cardiac ischemia-reperfusion injury and cardiac dysfunction.

Heart disease is also associated with the dysfunction of other organs such as the kidneys and liver. In this respect, some studies have identified serum bilirubin levels as a potential indicator of liver dysfunction following cardiac insults. For example, Lyu et al. have demonstrated that very high levels of serum bilirubin (> 3 mg/dL) following veno-arterial extracorporeal membrane oxygenation (ECMO) was associated with low in-hospital survival rate [34]. Very high serum bilirubin has been associated with poorer prognosis in patients with heart failure (HF) and serum bilirubin concentration could be an independent predictor of mortality and may improve risk stratification [35]. Thus, a therapeutic level of bilirubin needs to be established in order to maximize treatment efficiency. Significant hyperbilirubinemia due to underlying liver dysfunction may not be beneficial due to separate mechanisms which may be independent of the actions of bilirubin per se, but serum bilirubin levels could be an important marker of disease progression in cardiac patients.

Role of Bilirubin in Metabolic Protection

Serum UCB levels are negatively correlated with the degree of overweight and obesity in both men and women in extensive population studies [9•, 36•]. Serum UCB levels also negatively correlate with the development of type II diabetes in both men and women [37, 38]. In addition, moderate hyperbilirubinemia associated with Gilbert's polymorphism is

associated with lower body mass index (BMI), hip circumference (HC), fat mass, and lipid profile as compared to healthy controls and type II diabetic patients [8••]. Enhancing heme oxygenase-1 (HO-1) activity increases bilirubin that has been shown to reduce adiposity [39, 40] and recently found to function as a hormone by activating PPARα by direct binding [41••]. In obese mice, increasing HO-1 production of bilirubin reduces ROS and hepatic steatosis by increasing PPARα and FGF21 levels [42, 43].

Studies in a mouse model of Gilbert's have also shed light on the protective metabolic actions of moderate hyperbilirubinemia. Gilbert's mice were protected from dietary-obesity induced hyperglycemia and hyperinsulinemia [44•]. These mice are also protected against hepatic steatosis and exhibit elevated levels of serum fibroblast growth factor 21 (FGF21) and decreased expression of hepatic lipid synthesis genes such as fatty acid synthase (*Fasn*), sterol regulatory element-binding protein-1 (*Srebf1*), and acetyl-CoA carboxylase (*Acaca*) [44]. Further studies in both diet-induced obese (DIO) and db/db mice have demonstrated that bilirubin administration attenuates hyperglycemia and obesity [10, 45]. Although bilirubin signaling was not explicitly evaluated in these studies, UCB treatment was associated with the activation of insulin-signaling pathways and decreased levels of inflammatory cytokines as well as markers of endoplasmic reticulum (ER) stress [10]. Bilirubin has been shown to protect against several complications of diabetes, including diabetic nephropathy and retinopathy [46–48]. While most studies have demonstrated a protective effect of serum bilirubin on the development of type II diabetes and its complications, some studies have reported a detrimental effect of serum bilirubin [49] or reported a U-shaped relationship between serum bilirubin and the development of diabetic retinopathy [50]. However, reports show that serum bilirubin levels are lower in humans who are obese or diabetic [51•].

Alterations in plasma lipids and cholesterol levels can promote cardiovascular disease. Models of moderate hyperbilirubinemia in individuals with Gilbert's polymorphism as well as animal studies have demonstrated the effects on lipids and cholesterol, which contributes to the protective actions of UCB. Individuals with Gilbert's polymorphism have lower serum cholesterol, low-density lipoprotein (LDL), and oxidized LDL (ox-LDL) [52]. Similar effects on serum cholesterol and LDL levels were observed in both a mouse model of Gilbert's as well as DIO mice treated with UCB [44•, 45]. Bilirubin regulates cholesterol metabolism through several potential pathways one being its effects on the expression of the ATP-binding cassette transporter A1 (ABCA1) which is the primary transmembrane cholesterol transporter involved in apolipoprotein A1-mediated cholesterol efflux [53].

Apart from its role in the reduction of biliverdin to bilirubin, BVRA also has unique domains which allow it to also interact with AKT, protein kinase C (PKC), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and insulin receptor pathways [1]. Several studies have demonstrated that the C-terminal peptide, KYCCSRK, of human BVRA can serve as stimulators of insulin receptor kinase (IRK) and glucose uptake as well as inhibitors of IRK and glucose uptake and that administration of this peptide markedly improved glucose uptake in type II diabetic *ob/ob* mice [54]. The loss of the BVRA isoform in the liver resulted in marked hepatic steatosis and decreased in liver glycogen storage in response to a high-fat diet [4]. Hepatocyte-specific loss of BVRA also

decreases fat burning genes such as *Fgf* 21 and carnitine palmitoyltransferase 1A (*Cpt1a*) [4]. The importance of BVRA in the protection against steatosis is further highlighted in mouse kidney proximal tubule cells in which the BVRA gene was deleted using CRISPR/Cas 9 technology. BVRA-deficient proximal tubule cells exhibit increased steatosis and ROS generation, and the loss of mitochondrial function, which induced lipoapoptosis [55]. The loss of hepatocyte BVRA increased the activity of glycogen synthase kinase-3β (GSK3β) activity by a reduction in Ser9 phosphorylation, which is inhibitory. BVRA regulates hepatic GSK3β activity through Akt-mediated increases in Ser9 phosphorylation [56, 57]. The rise in GSK3β activity due to the loss of BVRA-mediated Ser9 phosphorylation increased Ser(P)^{73} PPARα resulting in ubiquitin-mediated degradation and decreased activity of PPARα.

New Function of Bilirubin as a Signaling Molecule

The mode of action of bilirubin has been attributed to its potent antioxidant effects. However, recent studies have found that bilirubin is much more than an antioxidant and can also function as a hormone [51•]. The structure of unconjugated bilirubin shares partial similarity to other known peroxisome proliferator-activated receptor α (PPARα) ligands such as WY 14,643 and fenofibrate [41]. Compounds that target the PPARs may simultaneously activate all three PPARs (PPAR pan agonists) or can have selective modulation of a single PPAR such as PPARα so-called selective peroxisome proliferator-activated receptor modulators (SPPARM) [58]. Ligands that bind to PPARα confer a conformational change to the protein resulting in enhanced DNA binding to PPAR response elements (PPREs) in promoters and enhancers of regulated genes. Recent studies have demonstrated that both biliverdin (bilirubin precursor) and bilirubin can activate a PPARα-dependent reporter gene in transiently transfected COS-7 cells expressing PPARα; however, only UCB can tightly bind to PPARα [41••]. Suppression of PPARα via shRNA in human HepG2 hepatocytes showed that it mediates 95% of bilirubin's transcriptome responses [59••]. Interestingly, when comparing known PPARα ligands WY 14,643 and fenofibrate, biliverdin increased the PPARα-dependent reporter gene to the same extent as fenofibrate [41]. Fibrates are drugs classically given to treat hyperlipidemia and have also been reported to have beneficial cardiovascular effects in non-human primate models [60]. These results indicate that bilirubin is an endogenous activator of PPARα with activity similar to the known PPARα agonist fenofibrate.

There is also strong evidence from in vivo studies which demonstrated that bilirubin acts as a SPPARM. Bilirubin treatment results in decreases in body weight, body fat, fasting blood glucose, and increases hepatic and serum FGF21 in wild-type but not in PPARα knockout mice [41••]. Gilbert's mice that have moderate hyperbilirubinemia due to expression of the mutated form of human UGT1A1 exhibit enhanced hepatic levels of total PPARα protein and decreased levels of phosphorylated serine 73 (SerP^{73}) PPARα as compared to wild-type mice fed a high-fat diet [44•]. As noted above, Ser(P)^{73} is the primary site which PPARα is targeted for ubiquitin-mediated degradation; thus, lowering phosphorylation at this site would decrease PPARα turnover and increase its activity [4]. The decrease in Ser(P)^{73} PPARα and increased activity was reflected by elevated expression of several PPARα target genes such as *Fgf21* and *Cyp4A* in the liver of humanized UGT1A1*28 mice as compared

to wild-type mice [44•]. However, the mechanism by which bilirubin decreases Ser(P)^{73} PPARα is not currently known but may be mediated through regulation of glycogen synthase kinase-3β (GSK3β) in a mechanism similar to that of BVRA. Lastly, RNA sequencing analysis in human liver HepG2 cells under control conditions or when PPARα was inactivated via lentiviral expression of shRNA demonstrated an overwhelming dependence of PPARα on the ability of bilirubin to activate gene expression (398 vs. 23 genes) [59••]. This data demonstrates that the transcriptional effects of bilirubin are PPARα-dependent while its other functions only regulate a small set of genes.

Bilirubin activates PPARα in the mildly elevated range (10–50 μM), but other studies have found that bilirubin at very high jaundice levels activates other receptors as well. Patients with cholestatic jaundice, a condition associated with extremely high levels of bilirubin, commonly report experiencing an intense itch. Recent studies by Meixiong et al. have demonstrated that bilirubin binds and activates two Mas-related G protein-coupled receptors (MRGPR), mouse MRGPRA1 and human MRGPRX4 found on sensory neurons [61]. These studies demonstrated that bilirubin is only able to activate these receptors at very high levels (> 150 μM), which are well above the physiological levels of bilirubin. Furthermore, patients with genetic forms of hyperbilirubinemia such as Crigler-Najjar Type 1 or Dubin-Johnson syndrome rarely complain of itch although their serum bilirubin levels are well within the range of bilirubin to bind to the MRGPR. The reason for this is unknown but may be due to alterations in other serum factors in cholestatic jaundice which contribute to the binding of bilirubin to MRGPR or the ability of unbound bilirubin to get to the skin in individuals with genetic hyperbilirubinemia. It is now becoming clear that bilirubin is much more than a bile pigment or an antioxidant but can act as a signaling molecule to regulate many different physiologic functions.

Conclusion

Bilirubin has numerous health benefits on both the cardiovascular system and metabolism (Fig. 1). Recent studies have revealed novel functions of bilirubin as a signaling molecule capable of activating nuclear hormone receptors as well as MRGPR. These new signaling actions of bilirubin need to be further explored and characterized in future preclinical studies utilizing models in which bilirubin effector targets are altered. While the results from large population studies demonstrate a negative relationship between serum bilirubin levels and the development of cardiovascular and metabolic diseases, these findings have yet to be translated to help at-risk patient populations. Specific strategies to increase serum levels of unconjugated bilirubin need to be developed and tested in appropriate preclinical models and then translated so that individuals most at risk for the development of cardiovascular and metabolic diseases can take advantage of the many healthy beneficial actions of bilirubin.

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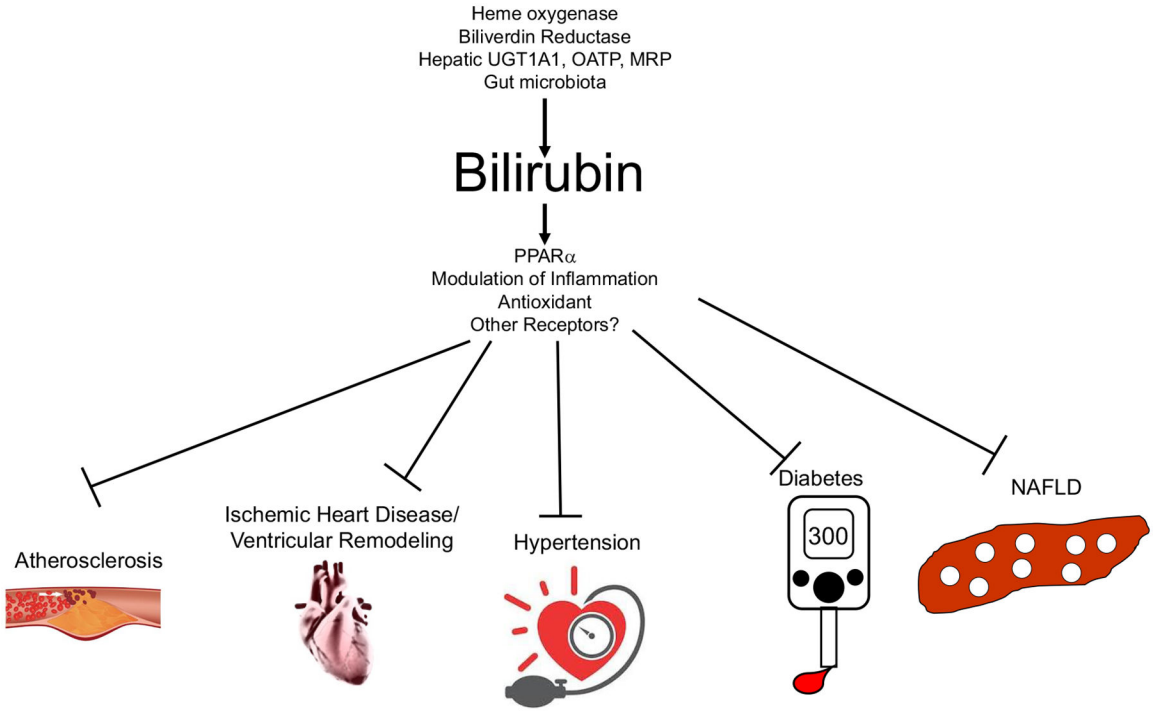

Fig. 1.

Summary of protective actions of bilirubin. Serum bilirubin levels are regulated by heme oxygenase, biliverdin reductase which is primarily responsible for the generation of bilirubin. Hepatic UDP-glucuronosyltransferase-1 (UGT1A1), organic-anion-transporting polypeptide (OATP), and multidrug resistance proteins (MRP) regulate the levels of serum bilirubin by regulation of the conjugation and transport of bilirubin. Gut bacteria are also responsible for the elimination of bilirubin as well. Bilirubin then acts through multiple pathways, including signaling through peroxisome proliferator-activated receptor-α (PPARα), regulation of inflammation, as well as antioxidant function to exert its cardiorenal and metabolic protection