Role of Serum Bilirubin in Coronary Artery Disease

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ABSTRACT

Lipid oxidation and formation of oxygen radicals are important elements of arterial plaque formation and atherosclerosis, and are involved in the pathophysiology of coronary artery disease (CAD). Since bilirubin has antioxidant properties, it has been suggested that it may have a protective role in the atherosclerotic process by preventing formation of oxidized LDL. The antioxidant capacity of bilirubin and its ability to provide potent scavenging effect of peroxyl radicals have led to suggest that mild increase in the circulatory bilirubin may have a physiologic role to protect against the disease processes that involve oxygen and peroxyl radicals. This review briefly examines the role of bilirubin and its protective function against the coronary artery disease.

KEYWORDS: Bilirubin, Antioxidant activity, Cardiovascular disease

INTRODUCTION

Bilirubin, the principle bile pigment, is the end product of heme catabolism. For many years, bilirubin was considered as toxic waste product formed during heme catabolism. However, more recent evidence suggests that bilirubin is a potent physiological antioxidant that may provide important protection against the atherosclerosis, coronary artery disease (CAD), and inflammation. Further, the development of CAD involves lipid oxidation and formation of oxygen radicals and that atherosclerosis and inflammation are associated with formation of oxygen and peroxyl radicals [1-3].

Bilirubin as physiologic Antioxidant:

Several studies have found that different circulating forms of bilirubin are powerful antioxidants, viz. free bilirubin, albumin-bound bilirubin, conjugated bilirubin, and unconjugated bilirubin were all found to be effective scavengers of peroxyl radicals and able to protect human...
LDL (low density lipoprotein) against peroxidation [4-8].

On the basis of the known involvement of oxidized LDL in the formation of atherogenic plaques and the ability bilirubin to serve as a potent lipid chain-breaking antioxidant under physiological conditions, it was suggested that the increased physiological concentrations of plasma bilirubin may reduce atherogenic risk [9,10]. Both animal and human studies have substantiated the suggestion that bilirubin is a physiological antioxidant. Yamaguchi and co-workers [11,12] isolated and identified oxidative metabolites of bilirubin (biotripyrrins) from the urine of healthy humans and ascorbic acid-deprived rats treated with endotoxin. Feeding of ascorbic acid, a documented physiological antioxidant, reduced the secretion of bilirubin metabolites and suppressed the endotoxin-stimulated hepatic concentration of HO (heme oxygenase) mRNA [12].

Several studies have noted an inverse relationship between the incidence of CAD and circulatory total bilirubin [13,14]. This important finding indicated that a lower than normal serum bilirubin concentration is associated with the presence of ischemic heart disease [13] and patients with early familial CAD have an average total serum bilirubin of 8.9 ± 6.1 µmol/L compared with 12.4 ± 8.1 µmol/L in healthy control subjects [14].

A prospective study in middle-aged British men, Breimer et al. [15] observed a U-shaped relationship between circulating bilirubin concentrations and cardiovascular risk, leading to the conclusion that low concentrations of serum bilirubin are associated with increased risk of ischemic heart disease. These and other investigators found that plasma bilirubin correlated inversely with several known risk factors for CAD, such as smoking, LDL-cholesterol, diabetes, and obesity, and correlated directly with the protective factor HDL-cholesterol [16,17]. The reduced concentration of total bilirubin in plasma is related univariately and multivariately to the presence of CAD, and this relationship remained significant after adjustment for known CAD risk factors such as age, cholesterol, HDL-cholesterol, smoking, and systolic blood pressure [16].

On the basis of these findings, low bilirubin was suggested as an independent risk factor for CAD, and an inverse correlation was demonstrated between bilirubin concentration and CAD morbidity. Further support for the existence of this inverse correlation came from the work of Hunt et al. [18], who described a genetic variation in bilirubin concentration, with individuals with early CAD displaying lower bilirubin than unaffected persons.

It would be interesting to determine which of the different entities of circulating bilirubin possesses cardioprotective capacity and is associated with the reduced risk for CAD. Antioxidant activity and cardioprotective potential may be attributed to any of the bilirubin forms, including free unconjugated bilirubin, protein-bound unconjugated bilirubin, delta bilirubin, or mono-/diconjugated bilirubin[7,10,19]. Under physiological conditions, the predominant circulatory form of bilirubin is the unconjugated, albumin-bound form. It is not known whether conditions that modify the relative proportions of this form of bilirubin in the blood, i.e., protein binding, acidosis, hypoxia, and extent of hemolysis, affect the cardioprotective potential of bilirubin. Protein binding, which is modulated by changes in plasma albumin concentrations, the concentrations of drugs that compete on binding, acidosis, and other factors, is expected to affect the balance between free (diffusible) and bound unconjugated bilirubin and thereby change the penetration of unconjugated bilirubin into cells. Likewise, changes in membrane integrity that are induced by hypoxia could potentially modulate the bilirubin transfer capacity of the membrane. In view of these complex interactions, it is important to establish the antioxidative capacity of the different bilirubin forms and to assess how the circulating concentration of free bilirubin, circulating albumin, blood pH, and the presence of drugs modulate bilirubin antioxidative capacity.
The proposed mechanism for antioxidant activity of Bilirubin:

Bilirubin can scavenge the chain-carrying peroxyl radical by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical Bil*

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\text{LOO}^* + \text{Bil} \rightarrow \text{LOOH} + \text{Bil}^* \\
\text{Bil}^* + \text{LOO}^* \rightarrow \text{Bil-OO} \\
\text{Bil}^* + \text{O}_2 \leftrightarrow \text{Bil-OO}^* \\
\text{LOO}^* + \text{BV} \rightarrow \text{LOO-BV}^* \\
\]

Both unconjugated bilirubin (BU) and conjugated bilirubin (BC) can serve as antioxidants, protecting human LDL from lipid peroxidation in vitro against peroxyl radicals (generated by 2,2’-azobis [2-amidinopropane] dihydrochloride). At concentrations as low as 10nM, Bilirubin can protect against 10,000-fold greater concentrations of H$_2$O$_2$. Under physiologic conditions, bilirubin provides more potent protection against lipid peroxidation than α-tocopherol, formerly known to be most effective in preventing lipid peroxidation [20].

Recent research indicates that bilirubin may be the most abundant endogenous antioxidant in mammalian tissues. A linear relationship (R$^2$ = 0.99) has been identified between plasma antioxidant capacity and unconjugated bilirubin concentration in newborn infants. This both confirms bilirubin’s significance as a plasma antioxidant and suggests that moderate increases in plasma bilirubin might be favourable to infants under oxidative stress. A cytoprotective enzyme that breaks the prooxidant molecule heme into biliverdin (immediately converted into bilirubin), iron, and carbon monoxide. HO-2 (Heme Oxygenase-2), the constitutive isofrom, is highly active in neurons and accounts for most of the HO activity in the brain. Destroying the HO-2 gene and thus limiting BR production, leads to increased oxidative damage following cerebral ischemia [21].

Low serum bilirubin has been shown to be strongly correlated with several cardiovascular risk factors, including age, cigarette smoking, social class, diabetes, serum cholesterol, lower FEV$_1$, and lower serum albumin. Serum bilirubin was found to have a U-shaped relationship with the events of ischemic heart disease (IHD). Bilirubin perfusion was shown to decrease infarct damage significantly caused by IHD. Serum bilirubin concentrations in the upper range of normal values protect against the coronary artery disease (CAD)[22].

However, concentrations in the lower range increase the atherogenic risk and thus risk of IHD. Schwertner et al, found an unexpected inverse association between serum total bilirubin and CAD. The strength of the association with CAD was similar to that of smoking or of systolic blood pressure.

**Plausible Mechanisms of Bilirubin Action in Prevention of Atherosclerosis:**

Several mechanisms have been suggested to play a potential role in the antiatherogenic and cardioprotective effects of bilirubin.

**a) Bilirubin-mediated inhibition of lipid oxidation**

Lipoproteins, particularly LDL, are highly susceptible to oxidation, and it is known that the atherogenic process involves uptake of oxidized LDL by intimal macrophages, leading to the accumulation of lipid-rich foam cells. Given the antioxidant capacity of bilirubin, it is plausible that bilirubin protects lipids and lipoproteins against oxidation and thereby offers protection against atherogenesis. Accordingly, low bilirubin concentrations may be associated with increase in the oxidized lipids and lipoproteins and therefore, with enhanced atherogenic plaque formation [13,23].

**b) Bilirubin as reflection of enhanced HO (Heme Oxygenase) activity**

Increased HO activity may account for the antiatherogenic and cardioprotective effects of bilirubin through increased elimination of heme and/or enhanced production of CO (Carbon Monoxide), iron, and biliverdin. Changes in the concentration of any of these metabolites could affect the pathophysiology of atherosclerosis [5,24]. For example, HO-1-mediated consumption of heme may reduce heme-induced toxic cell injury, and decreased hemoglobin concentrations may enhance vasodilatation. Furthermore,
hemoglobin is a scavenger of NO (Nitric oxide) that blunts NO-dependent vasodilatation. CO could affect cardiovascular function through activation of soluble guanylate cyclase and the consequent increase in the intracellular cGMP concentrations. CO is also an active vasodilator involved in the regulation of vasomotor tone, platelet aggregation, and vascular smooth muscle cell proliferation [27,29].

Another potential mechanism explaining the association between HO activity and CAD risk may be related to the ability of HO-1 to release iron and change the concentrations of iron stores [13,9,26]. Mice lacking functional HO-1 develop an anemia associated with abnormally low serum iron concentrations and accumulate iron in the liver and kidney. These iron stores may contribute to tissue injury and chronic inflammation associated with the oxidative damage that characterizes HO-1-deficient animals [27]. The induction of HO by heme is associated with increased expression of ferritin [28]. On the basis of this finding it was suggested that iron released through HO activity drives the synthesis of ferritin and that ferritin, by virtue of its iron-binding capacity, provides protection to endothelial cells against oxidative damages [28].

c) Immune reactions and inflammatory processes

The involvement of bilirubin in immune reactions and inflammatory processes has also been documented. Nakagami et al. [29] noted that biliverdin and bilirubin inhibit complement-dependent reactions in vitro and that biliverdin administration inhibits Forssmann anaphylaxis in guinea pigs. On the basis of these findings it is possible that bile pigments are endogenous tissue protectors by virtue of their anticomplement activity [29]. A correlation between bilirubin metabolism and inflammatory processes is also supported by observations that high HO activity is linked to a faster resolution of inflammation, whereas inhibition of this enzyme appears to potentiate the inflammatory responses [30].

Both endothelial and inflammatory cells [31] express all of the necessary enzymes involved in bilirubin synthesis and degradation, implies a high degree of regulation of cellular bilirubin levels and supports a potentially broad role for this endogenous bile pigment as a physiological regulator of inflammation. VCAM-1-(Vascular cell adhesion molecule-1) mediated leukocyte recruitment is implicated in the pathogenesis of a number of inflammatory conditions, including atherosclerosis [32,33] and inflammatory bowel disease [34 - 36]. With regard to the former, VCAM-1 is detectable in atherosclerotic plaques [37] and endothelial expression of this adhesion molecule has been shown to be an early event at sites predisposed to atherosclerosis [38]. Moreover, disruption of VCAM-1 expression in the atherosclerosis-prone low density lipoprotein receptor knockout mouse is associated with a significant decrease in the number of vascular lesions. Consistent with the hypothesis that bilirubin may modulate the process of atherogenesis by inhibiting VCAM-1 signaling, a host of epidemiological analyses have identified an inverse correlation between serum bilirubin levels and both the risk and severity of cardiovascular disease [39 – 42].

CONCLUSION

The distinct inverse correlation between plasma bilirubin concentration and CAD morbidity may have an important clinical and diagnostic implication. The clinical relevance relates to potential preventive and therapeutic approaches, whereas the diagnostic relevance stresses the plasma bilirubin concentration as a provisional new marker of atherogenic risk that can be measured easily in the clinical laboratory and applied in medical practice.

FUTURE SCOPE

Although the mechanism(s) underlying the ability of circulating forms of bilirubin concentrations in protecting against the CAD remain to be clarified, it is possible to say that either the bilirubin concentration itself or changes in the concentrations of other component(s) in the bilirubin synthetic pathway are involved in the protective action. These additional components may include heme, biliverdin, CO, and iron, which are regulated by the activity of HO and have all been implicated in the physiology and pathology of the cardiovascular system. HO is expressed at
low basal concentrations in vascular endothelial and smooth muscle cells and is induced by oxidative stress, inflammatory mediators, and oxidized LDL. The complex interactions between HO expression, the circulating concentrations of its substrate and products, and the effect of these components, and specifically of bilirubin, on the vasculature, on lipid metabolism, and on the cardiovascular system will hopefully be the focus of extensive research in the coming years.

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