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Review

Bilirubin, platelet activation and heart disease: A missing link to cardiovascular protection in Gilbert's syndrome?

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ABSTRACT

Gilbert's syndrome (GS) is a relatively common condition, inducing a benign, non-hemolytic, unconjugated hyperbilirubinemia. Gilbert's Syndrome is associated with mutation in the Uridine Glucuronosyl Transferase 1A1 (UGT1A1) gene promoter, reducing UGT1A1 activity, which normally conjugates bilirubin allowing its elimination from the blood. Individuals with GS demonstrate mildly elevated plasma antioxidant capacity caused by elevated levels of unconjugated bilirubin (UCB), reduced thiols and glutathione. Interestingly, the development of, and risk of mortality from, cardiovascular disease is remarkably reduced in GS individuals. An explanation for this protection may be explained by bilirubin's ability to inhibit multiple processes that induce platelet hyper-reactivity and thrombosis, thus far underappreciated in the literature. Reactive oxygen species are produced continuously via metabolic processes and have the potential to oxidatively modify proteins and lipids within cell membranes, which may encourage the development of thrombosis and CVDs. Oxidative stress induced platelet hyper-reactivity significantly increases the risk of thrombosis, which can potentially lead to tissue infarction. Here, we discuss the possible mechanisms by which increased antioxidant status might influence platelet function and link this to cardiovascular protection in GS. In summary, this is the first article to discuss the possible role of bilirubin as an anti-thrombotic agent, which inhibits platelet activation and potentially, organ infarction, which could contribute to the reduced mortality rate in mildly hyperbilirbinemic individuals.

1. Introduction

Platelet hyperactivity plays a pivotal role in the development of thrombosis and cardiovascular diseases (CVD). Oxidative stress, inflammation and endothelial dysfunction are considered major risk factors for increased platelet activity. These risk factors act synergistically to induce platelet activation and coagulation, thereby significantly increasing the risk of thrombosis and CVD. Hence, platelet function has evolved as an important marker for predicting future CVD risk, especially in individuals with co-morbidities including obesity and type 2 diabetes mellitus (T2DM) [1]. According to recent World Health Organization reports, an estimated 17.3 million deaths annually are attributed to CVD, approximating 30% of all deaths (~7.3 million due to CHD and 6.2 million due to stroke) and is predicted to continue rising in the coming years [2–4]. Increased free radical production and oxidative stress is emerging as a key risk factor in the development of atherosclerosis and CVD [5]. An imbalance between free radical production and the body's endogenous antioxidant defence system to scavenge them leads to oxidative stress and potentially, oxidative damage to cellular macromolecules [6]. Oxidative stress induced by auto-oxidation of glucose leads to further production of reactive oxygen species, lipid peroxidation, inflammation, endothelial dysfunction, smooth muscle proliferation, matrix metalloproteinase activation and reduced nitric oxide (NO) bioavailability, and therefore could play an important role in the initiation and progression of atherosclerosis (see Fig. 1) [5,7,8]. Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), when generated in large quantities and in the context of platelet function, can mobilize intravascular arachidonic acid (AA) via activation of phospholipase A_2 (PLA_2). Such activation stimulates calcium (Ca^{2+}) dependent and independent signaling pathways, thus encouraging platelet hyper-activation and subsequent thrombosis, leading to atherothrombosis, embolus and organ ischemia [1].

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Recent studies have revealed new biological roles for UCB, including potent antioxidant, anti-mutagenic, immune-modulatory, platelet inhibitory and possibly lipid lowering effects [9–14]. This is in contrast to its well known, potentially cytotoxic property, in neurons [15]. Hence, Gilbert's syndrome (GS), a condition of mild and benign hyperbilirubinemia has recently become a topic of great interest because it is clearly associated with protection from CVD [12,16–18]. Evidence from a recent meta-analysis supports the above statement, showing that mildly elevated UCB, as seen in GS, is negatively associated with the risk of developing CVD, which might be related to inhibition of free radical lipid and low density lipoprotein (LDL) oxidation [16,19]. Unconjugated bilirubin scavenges free radicals in *in vitro* and *in vivo* models [12,16–18]. Furthermore, elevated serum UCB in GS increases circulating antioxidant capacity, which might delay the process of atherosclerosis [12]. Large epidemiological studies indicate that individuals with GS experience reduced rates of CVD, cancer and all-cause mortality [20–22]. Furthermore, in a longitudinal study, Horsfall and colleges show a striking 50% reduction in all-cause mortality in GS, when compared to individuals without GS [23]. Therefore, GS represents an invaluable and clinically relevant translational model to test the physiological importance of bilirubin in preventing disease.

Gilbert's syndrome (also known as Gilbert-Meulengracht syndrome) is a benign condition affecting 3–10% of the population and is caused by a genetic polymorphism in the promoter region of the uridine diphosphate glucuronosyltransferase (UGT1A1) gene. This mutation reduces hepatic UGT1A1 enzyme synthesis, bilirubin conjugation and excretion [24–26] and is therefore, associated with a mild and chronically elevated serum UCB concentration in GS (>17 μmol/L) [25,26].

1.1. Risk factors for thrombosis

Elevated body mass index (BMI), glucose concentrations, total cholesterol, hypertension, alcohol and tobacco use are considered major risk factors of CVD and metabolic disorders [27]. Auto-oxidation of glucose is believed to induce oxidative stress by increasing ROS production [28,29]. Elevated oxidative stress, especially in uncontrolled type 2 diabetes, can significantly increase the risk of cardiovascular complications and elevates the risk of platelet hyperactivity by: (i) increasing the production of F2-isoprostane (8-epiprostaglandin F2α) which can amplify platelet response to agonists, (ii) reduced endothelial nitric oxide synthase (eNOS) activity and nitric oxide (NO) production (iii) increasing platelet receptor signaling due to oxidative stress [30]. With increasing resistance to existing anti-platelet therapy, the need for effective alternative therapies is of clear importance. Antioxidants, which inhibit platelet hyper-activation, are products of renewed interest due to their antithrombotic potential [13,30–33].

The antioxidant properties of bilirubin are generally underappreciated, however, these properties could explain its platelet inhibitory effects [13]. Individuals with GS, also have reduced levels of P-selectin, CD 40 ligand (CD40L), inflammatory biomarkers and have improved endothelial function, which together indicate

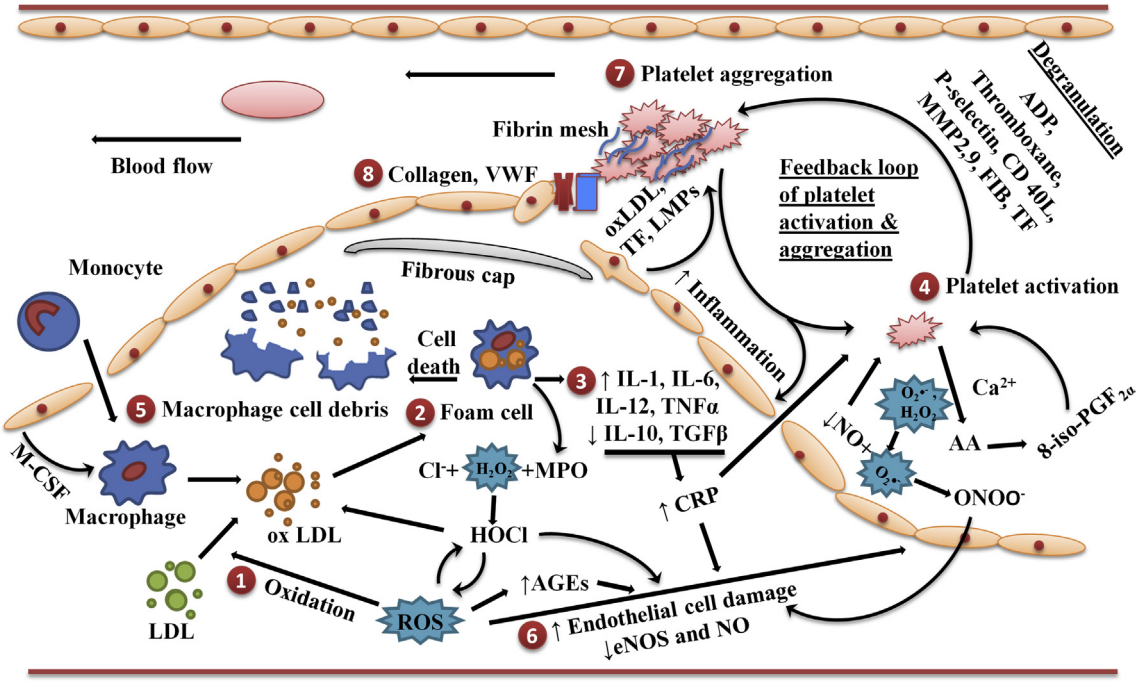

Fig. 1. Pathogenesis of ROS induced atherosclerosis. 1) Increased production of oxidized low density lipoprotein (oxLDL) by ROS induces endothelial dysfunction, recruitment of macrophages and inflammation; 2) phagocytosis of oxLDL by macrophages; 3) release of inflammatory cytokines and MPO; MPO in the presence of H_2O_2 and Cl^- forms HOCl, which can induce oxidative modification to LDL, induce endothelial dysfunction and further produce ROS; 4) Platelet activation due to inflammation, ROS and endothelial dysfunction leads to platelet degranulation and production of other oxidants; $O_2^{\bullet-}$ reacts with NO to produce $ONOO^-$ increasing platelet aggregation and endothelial dysfunction; H_2O_2 oxidizes AA and forms 8-iso-$PGF_{2\alpha}$; 5) macrophage apoptosis and cell debris promote the evolution of the atherosclerotic lesion; 6) intravascular inflammation, elevated CRP, AGE production and ROS induce endothelial dysfunction by scavenging eNOS and NO; 7) Agonists from degranulated platelets induce platelet aggregation and activate the coagulation cascade, thus forming a solid clot i.e. thrombus; 8) rupture of the endothelial cell lining at an atherosclerotic lesion exposes collagen and vWF causing the thrombus to bind to the site, recruit circulating platelets and block the blood vessel. AA, arachidonic acid; ADP, adenine diphosphate; AGEs, advanced glycation-end products; Ca^{2+}, calcium; CD40L, CD40 ligand; CRP, C-reactive protein; Cl^-, chloride; eNOS, endothelial nitric oxide synthase; FIB, fibrinogen; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; LDL, low density lipoprotein; LMP, leukocyte micro-particles M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteins; MPO, myeloperoxidase; NO, nitric oxide; $O_2^{\bullet-}$, superoxide anion; $ONOO^-$, peroxynitrite; oxLDL, oxidized low density lipoprotein; ROS, reactive oxygen species; TF, tissue factor; TGF β, transforming growth factor β; TNF, tumor necrosis factor.