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Bilirubin: Striking Gold in Diabetic Vasculopathy?

Vascular complications are the primary cause of morbidity and mortality and a major contributor to health care costs in patients with diabetes (1). While the etiology of vascular disease is multifactorial, endothelial dysfunction characterized by a decrease in nitric oxide (NO) synthesis from endothelial NO synthase (eNOS) and blunted endothelium-dependent vasodilation is a seminal feature in diabetes-associated vascular disease. Given the dramatic increase in the incidence of diabetes worldwide, there is an urgent need for new therapies that targets this cellular defect in diabetes.

Although long considered a toxic waste product of heme metabolism, the yellow pigment bilirubin is now recognized as an important vasoprotective molecule. Bilirubin is formed by the action of inducible heme oxygenase-1 (HO-1) or constitutively expressed HO-2, which degrades heme into biliverdin, carbon monoxide (CO), and iron (Fig. 1). Biliverdin is rapidly reduced to bilirubin by biliverdin reductase (BVR). Bilirubin is further processed in the liver, where it is conjugated with glucuronic acid by uridine diphosphate-glucuronosyltransferase A1A (UGT1A1) to a water-soluble form for elimination. While early work identified the potent antioxidant properties of bilirubin, more recent studies found that bilirubin elicits anti-inflammatory and antiapoptotic effects in endothelial cells and improves endothelium-dependent vasodilation in murine hypercholesterolemic blood vessels (2–5). Clinical studies also support a protective role, as serum bilirubin concentrations show an inverse relationship with risk for a constellation of cardiovascular disorders (6). Interestingly, serum bilirubin levels are lower in patients with diabetes (7), and low serum values were identified as a crucial predictor of cardiovascular disease in specific populations with diabetes (8). Moreover, diabetic patients with Gilbert syndrome, who have moderately elevated levels of circulating bilirubin, have a lower rate of vascular complications than patients without the syndrome, further highlighting the potential importance of this pigment in diabetes (9).

In this issue, Liu et al. (10) use a highly integrated and complementary experimental approach to convincingly show that bilirubin corrects endothelial dysfunction in diabetes. In initial experiments, they demonstrate that pharmacological induction or adenoviral transfer of HO-1 improves endothelium-dependent vasorelaxation in both genetic and dietary mouse models of type 2 diabetes. Ex vivo induction of HO-1 also augments endothelium-dependent relaxation of diabetic blood vessels. However, the vascular benefit of HO-1 overexpression is lost when BVR is silenced, indicating that conversion of biliverdin to bilirubin is necessary for this response. A remarkable finding in the study is that serum concentrations of unconjugated bilirubin are reduced by nearly 80% in diabetic mice. Importantly, administration of unconjugated bilirubin to diabetic animals or isolated blood vessels from diabetic mice or patients with diabetes restores endothelial function. Finally, Liu et al. (10) identify protein kinase B (Akt) as the principal downstream target of bilirubin. They show that the activity of Akt and eNOS and levels of nitrite, a metabolite of NO, are reduced in aortas of diabetic mice, and this is corrected by bilirubin. However, this improvement is prevented by Akt inhibition. Comparable results are also obtained when cultured endothelial cells are exposed to high concentrations of glucose to simulate the in vivo conditions of diabetes. Collectively, these novel findings implicate a bilirubin deficiency in diabetes and demonstrate that raising bilirubin levels either through endogenous synthesis or exogenous application improves endothelial dysfunction in diabetes by activating Akt. These findings also extend recent work showing that bilirubin augments endothelial function in diabetic rats (11).

Consistent with earlier reports (12,13), Liu et al. (10) note that HO-1 induction moderately improves insulin sensitivity in diabetic mice. Although bilirubin was not tested, it likely mediates the metabolic action of HO-1 as bilirubin has been shown to reduce insulin resistance in the same mouse model (14). Intriguingly, CO fails to
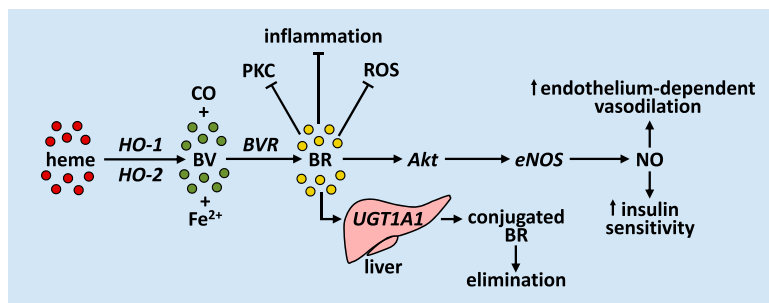

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Figure 1—Schematic diagram depicting the metabolism of heme and the beneficial actions of bilirubin in diabetes-induced endothelial dysfunction. Bilirubin, generated by the concerted action of HO-1 and BVR, leads to the sequential activation of Akt and eNOS and synthesis of NO, which improves endothelium-dependent vasodilation and insulin resistance. These salutary actions are further amplified by the ability of bilirubin to inhibit PKC, inflammation, and oxidative stress, which are known mediators of endothelial dysfunction in diabetes. Bilirubin is removed from the circulation by the liver and metabolized by UGT1A1 to yield water-soluble conjugated bilirubin for elimination. BR, bilirubin; BV, biliverdin; Fe^{2+}, ferrous iron; ROS, reactive oxygen species.

restore endothelial function in diabetic blood vessels, even though this gas is known to activate Akt and eNOS in endothelial cells (15). This finding also contrasts with previous studies demonstrating that CO restores endothelium-dependent relaxation in diabetic rats (11,16), indicating the need for further investigation in this area.

The study by Liu et al. (10) represents an important advance in the field, and similar to many good studies, it raises some interesting questions. In particular, how does bilirubin activate Akt? It is speculated that bilirubin may indirectly activate the kinase by suppressing oxidative stress and protein kinase C (PKC) activity, which are elevated in diabetes (17). This notion is reasonable but will require testing. Furthermore, what causes the precipitous decline in circulating unconjugated bilirubin in diabetes? Although vascular HO-1 levels are similar between control and diabetic animals, global induction of HO-1 in diabetic animals largely restores serum bilirubin, suggesting possible deficits in HO-1 in other tissue compartments. Alterations in HO-2, BVR, and/or UGT1A1 activity may also contribute to lower bilirubin levels in diabetes. While bilirubin ameliorates endothelial function in large conduit arteries, it is essential to extend this finding and confirm that endothelial dysfunction is also resolved in the microcirculation, as defects in these vessels contribute to diabetes-related organ pathologies. Finally, in order to translate these findings to the clinic, studies are needed to define an optimal range of the serum unconjugated bilirubin required to maintain endothelial function while averting possible toxic effects (18).

A schematic diagram depicting the beneficial actions of bilirubin in diabetes-induced endothelial dysfunction is shown in Fig. 1. In this model, bilirubin, generated via the concerted action of HO-1 and BVR, leads to the sequential activation of Akt and eNOS and synthesis of NO, which improves endothelium-dependent vasodilation and insulin resistance (19). Bilirubin also inhibits PKC activation, inflammation, and oxidative stress, which are known mediators of endothelial dysfunction. Several strategies

can be used to target bilirubin in diabetes. Endogenous circulating levels of bilirubin may be elevated using pharmacological or molecular approaches that induce HO-1 expression or suppress UGT1A1 activity. The feasibility of targeting UGT1A1 is supported by a report showing that atazanavir, a UGT1A1 inhibitor, raises unconjugated bilirubin levels and improves endothelial function in patients with diabetes (20). Alternatively, bilirubin or its precursor biliverdin may be directly administered. Future translational studies mining these approaches will reveal whether Liu et al. (10) have struck gold in managing diabetic vasculopathy.

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