CellPress

Opinion

A Novel Perspective on the Biology of Bilirubin in Health and Disease

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Unconjugated bilirubin (UCB) is known to be one of the most potent endogenous antioxidant substances. While hyperbilirubinemia has long been recognized as an ominous sign of liver dysfunction, recent data strongly indicate that mildly elevated bilirubin (BLB) levels can be protective against an array of diseases associated with increased oxidative stress. These clinical observations are supported by new discoveries relating to the role of BLB in immunosuppression and inhibition of protein phosphorylation, resulting in the modulation of intracellular signaling pathways in vascular biology and cancer, among others. Collectively, the evidence suggests that targeting BLB metabolism could be considered a potential therapeutic approach to ameliorate a variety of conditions.

From a Biological Waste Product to a Potent Biological Compound

UCB (see Glossary), the end product of the heme catabolic pathway, has long been recognized as a sign of liver dysfunction or a potential toxic factor causing severe brain damage in newborns. Mildly elevated BLB levels, as seen in patients with **Gilbert syndrome**, have been shown to be protective against an array of diseases associated with increased oxidative stress, such as cardiovascular diseases (CVD), diabetes and cancer [1,2]. These clinical observations are consistent with recent discoveries relating to how BLB might affect the pathophysiology of these diseases (Box 1). BLB is recognized as the most potent endogenous antioxidant due to its continuous recovery in the **BLB/biliverdin (BLV) redox cycle** (Figure 1), resulting in protective lipid peroxidation both *in vitro* [HEK293 cells in which biliverdin reductase (BLVR) was silenced]

Box 1. The Clinician's Corner

Until recently, BLB was considered as a waste product of heme with very limited biological activity. However, BLB has been described as a sign of liver disorders since Hippocrates. More recently, evidence suggests that BLB and related products in BLB metabolism (the yellow players) have a major role in several cellular events.

Together with uric acid, BLB is one of the most active antioxidant molecules in the human body. This property may explain some of its effects on cellular functions (growth, migration, differentiation, and modulation of the immune response).

When translated into humans, these activities may account for the reduced prevalence of CVD diseases, cancer, and metabolic syndrome in subjects with slightly higher serum BLB levels, as is the case of patients with Gilbert syndrome. Less clear and still under study is the possible relation between serum BLB levels and neurological diseases.

On these grounds, the pharmacological modulation of the yellow players (mainly resulting in increasing UCB intracellular concentration) may become a promising and effective therapeutic approach.

Trends

Historically known for its toxicity but recently recognized as a powerful protective molecule, BLB is gaining more attention due to its pleiotropic biomolecular effects and those of the enzymes involved in BLB metabolism (the 'Yellow Players').

Both heme oxygenase (HMOX) and biliverdin reductase (BLVR) (the main enzymes in BLB metabolism) act on numerous signaling pathways, with unsuspected biological consequences. The interconnections of such pathways highlight an incredibly complex biomolecular network. Yellow player molecules can have important physiological and pathological biological outcomes. Their still unexplored roles merit attention, offering the possibility of being targeted for therapeutic benefit.

The moderately high levels of UCB in the blood of patients with Gilbert syndrome are suggestive of the protective role of BLB in non-neurological pathologies (cardiovascular diseases, cancer, and metabolic syndrome).

Cells and tissues might actively maintain the intracellular homeostasis of BLB, with the yellow players being viewed as novel antioxidant mechanisms in a cell.

This new point of view might also be applicable to neurological diseases, where BLB levels are lower than in healthy subjects.

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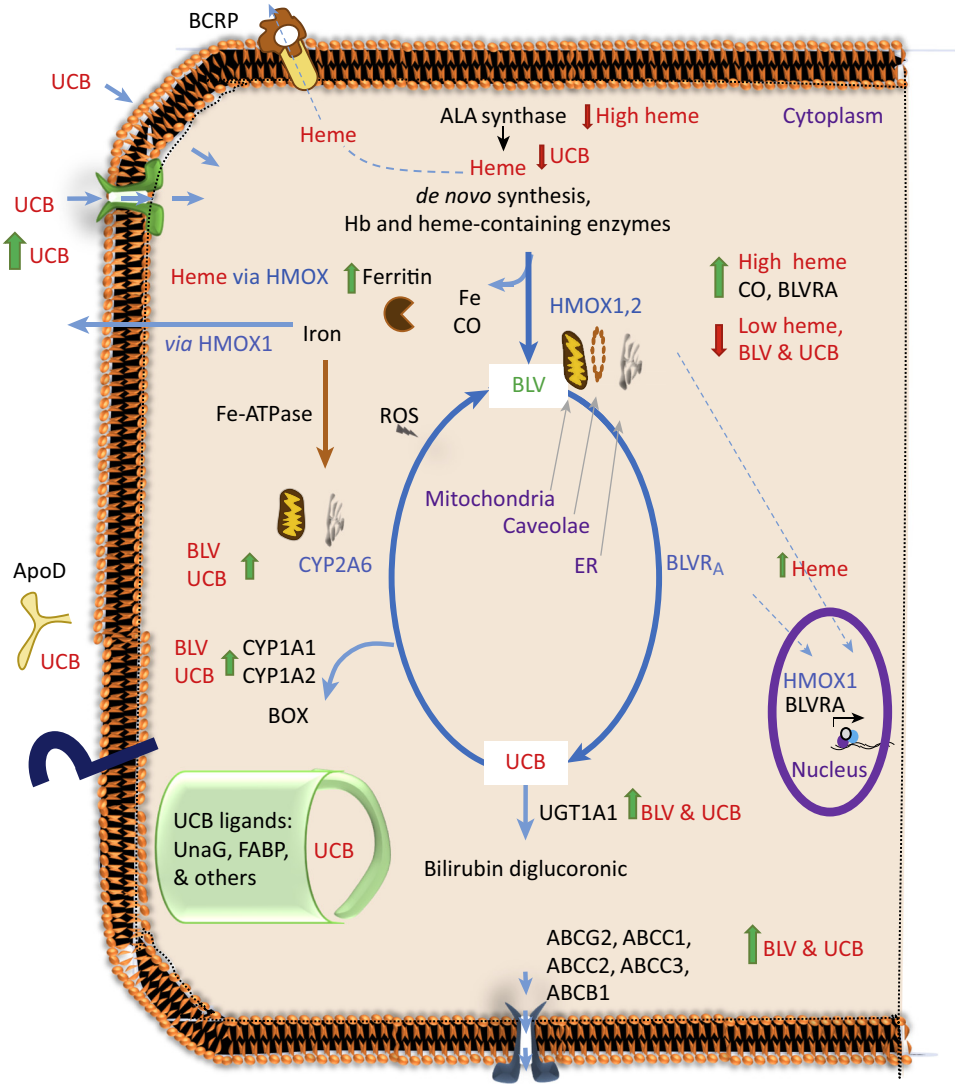

Trends in Molecular Medicine

Figure 1. Intracellular Bilirubin (BLB) Metabolism. Heme (produced by the action of the ALA: alanine synthase) [5-aminolevulinic acid (ALA)] is converted to biliverdin (BLV, plus CO, plus iron) [carbon monoxide (CO) (Fe)]by heme oxygenase (HMOX; localized in mitochondria, endoplasmic reticulum, and/or caveolae) 1 (inducible) and 2 (constitutive) enzymes. BLV is then converted to unconjugated bilirubin (UCB) by biliverdin reductase (BLVR) A. Thus, UCB may be produced either endogenously within the majority of the cells, or transported to the cell (typically hepatocytes): (i) by passive diffusion across the cellular bilayer due to its lipophilicity; or (ii) by apolipoprotein D (ApoD) expressed on the cell membraneor (iithe. BBLBalso UCB is stored inside the cell, bound to either (i) UnaG (belonging to the family of fatty acid-binding proteins, FABP); (ii) FABP1 (protein Z); (iii) lipids of the cellular membranes; or (iv) ligandin (glutathione S-transferase B = protein Y). UCB might be converted back to BLV by the activity of the cytochrome P450 mono-oxygenase 2A6 (CYP2A6), or during its oxidation by reactive oxygen species (ROS; sacrificial anode action). The intracellular UCB level might be reduced by either: (i) conjugation with glucuronic acid by the uridine-diphosphate glucuronosyl transferase 1A1 (UGT1A1), followed by (ii) efflux by ATP binding cassette (ABC) transporters G2, C2, C1, and B1; or (iii) oxidation by CYP1A1 and 1A2 (CYPs: localized in the mitochondria and/or endoplasmic reticulum) to bilirubin oxidation products (BOXes). Finally, the intracellular UCB level may be also regulated by export of heme, its precursor, out of the cell via breast cancer resistance protein (BCRP). All the enzymes involved in the UCB–BLV cycle are strictly interconnected and modulated (red arrows, inhibition; green arrows, induction). Both HMOX and BLVR might migrate into the nucleus and act as transcription factors.