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Metabolism of bilirubin and its biological properties

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SUMMARY

This overview is focused on the bilirubin molecule and its important biological properties. For a long time, bilirubin has been considered a waste product of heme catabolism with potential toxic effects especially on the central nervous system. However, bilirubin has attracted the attention of scientists because of its protective effects. Mildly elevated plasma bilirubin levels are associated with antioxidant, anti-inflammatory and immunomodulatory effects observed in patients with diseases associated with oxidative stress and chronic inflammation. The exact mechanisms of protective action of bilirubin have not been fully elucidated so far, although understanding of these mechanisms could facilitate the therapeutic use of substances that mildly increase bilirubin levels in the body.

Keywords: bilirubin, oxidative stress, inflammation, antioxidant effects.

SOUHRN

Valášková, P., Muchová, L.: Metabolismus bilirubinu a jeho biologické účinky

Článek se zabývá molekulou bilirubinu a jejími důležitými biologickými vlastnostmi. Bilirubin byl dlouhou dobu vnímán pouze jako odpadní produkt katabolismu hemu s potenciálně toxickými účinky zejména na centrální nervový systém. Dnes je však bilirubin v centru pozornosti vědců díky svým protektivním účinkům. Mírně zvýšené plazmatické koncentrace bilirubinu vykazují antioxidační, protizánětlivé a imunomodulační účinky, které byly pozorovány zvláště u pacientů s onemocněními souvisejícími s oxidačním stresem a chronickými záněty. Přesné mechanismy protektivního působení bilirubinu nejsou stále plně objasněny a jsou předmětem intenzivního výzkumu, přitom porozumění těmto mechanismům by mohlo pomoci terapeutickému využití látek mírně zvyšujících systémové koncentrace bilirubinu.

Klíčová slova: bilirubin, oxidační stres, zánět, antioxidační působení.

Bilirubin metabolism

Bilirubin (BR) is the end product of the heme degradation pathway in mammals. Firstly, heme is degraded by heme oxygenase to form biliverdin (BV), carbon monoxide and ferrous iron [1]. BV, a relatively polar and nontoxic compound, is further reduced by biliverdin reductase to BR. Conversion of BV to BR might be recycled back by oxidation BR to BV resulting in amplification of the effects of BR [2]. Since most of vertebrates produce only BV, it has been hypothesized that only BR, but not BV, could cross the placental barrier by diffusion due to its relative hydrophobicity. This effect is believed to prevent accumulation of BV in fetus [3].

BR is a symmetrical tetrapyrrole that consists of two rigid, planar dipyrroles joined by a methylene bridge (Fig. 1a). BR can exist in three isomers IIIα, IXα and XIIIα. Natural structure formed from heme metabolism is bilirubin IXα and this isomer is hydrophobic and virtually insoluble in plasma. The poor aqueous solubility of BR is due to its internal hydrogen bonding of its polar groups which are hidden from interaction with water molecules resulting in hydrophobic character (Fig. 1b) [4]. Because of these properties (low solubility at physiologic pH), BR must be solubilized by binding to a carrier molecule for its transport in the circulation. Physiologically most of BR (about 90 %) is non-covalently bound to albumin. Only a very small amount (<0.01 %) of BR is free, unbound (Bf), present in plasma in equilibrium with the albumin fraction, which is responsible for its pathophysiological effects on cells and tissues [5].

The transport of unconjugated BR (Fig. 2) is very important due to its toxicity when BR is accumulated in the cells. Unconjugated BR has to be transported to the liver, where a unique mechanism for its conjugation exists. Proposed mechanism of BR transport is a spontaneous transmembrane diffusion when unbound unconjugated BR and its albumin complex diffuse across the porous sinusoidal endothelium to reach the basolateral membrane of hepatocytes. Unconjugated BR is then rapidly transported into the liver cell most likely by the passive diffusion, although transporter proteins localized in the basolateral membrane of hepatocyte belonging to the group of the organic-anion transporting polypeptides (OATP) have been proposed to mediate its active transport as well [6]. In liver cells, unconjugated BR is temporarily solubilized by binding to two groups of organic-anion binding proteins (protein Y and protein Z), which is important for prevention of the passive reflux of UCB back into plasma and the binding of unconjugated BR to intracellular membranes, facilitating diffusion of unconjugated BR across the hepatocyte [7].

Like many other hydrophobic organic compounds that are poorly soluble, UCB is principally conjugated with endogenous glucuronic acid which is covalently at-

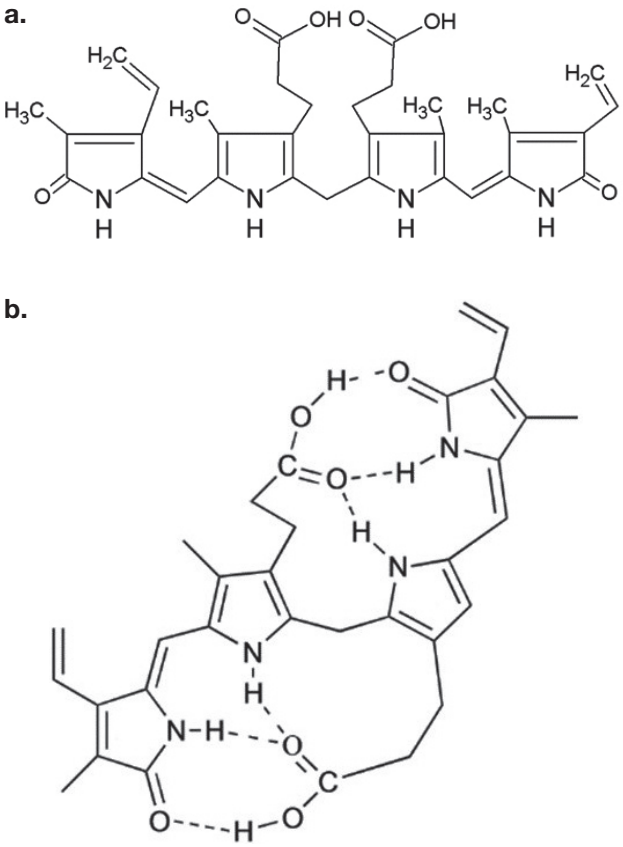

Fig. 1: Bilirubin structure. a Planar structure of bilirubin. b 3D structure of unconjugated bilirubin with intramolecular bonds.

tached to one or both COOH groups. This reaction occurs in smooth endoplasmic reticulum and makes BR conjugates water soluble. The conjugation is catalyzed by a specific microsomal hepatic bilirubin-UDP glucuronosyl transferase type I (UGT1A1) resulting in formation of monoglucuronosyl and bisglucuronosyl bilirubin [8]. BR conjugates are secreted against the concentration gradient through the canalicular membrane of hepatocyte into the bile by an active ATP-dependent transport mediated mainly by the multidrug resistance-associated protein 2 MRP2 (*ABCC2*) [9]. Latest research suggests that even under physiological conditions a part of conjugated BR is transported back to blood by multidrug resistance-associated protein 3 MRP3 (*ABCC3*) localized in the sinusoidal membrane. This substantial fraction could be subject to re-uptake via OATP (OATP1B1/3 in humans) again into hepatocytes. This process (so called "hepatocyte hopping cycle") probably prevents saturation of biliary extraction capacity of hepatocytes [10]. After secretion of conjugated BR into the bile, conjugated BR is poorly absorbed from the small intestine but undergoes limited hydrolysis to UCB by β-glucuronidases released from enterocytes and coliform bacteria. Intestinal bacteria in the distal ileum and colon degrade BR into urobilinogens, a group of colorless tetrapyrroles that are further oxidized to urobilins contributing to a coloration of normal urine and stool [11]. Part of the urobilinoid pool undergoes enterohepatic circulation, is absorbed from the intestine to the portal tract and re-secreted by the liver into the bile.

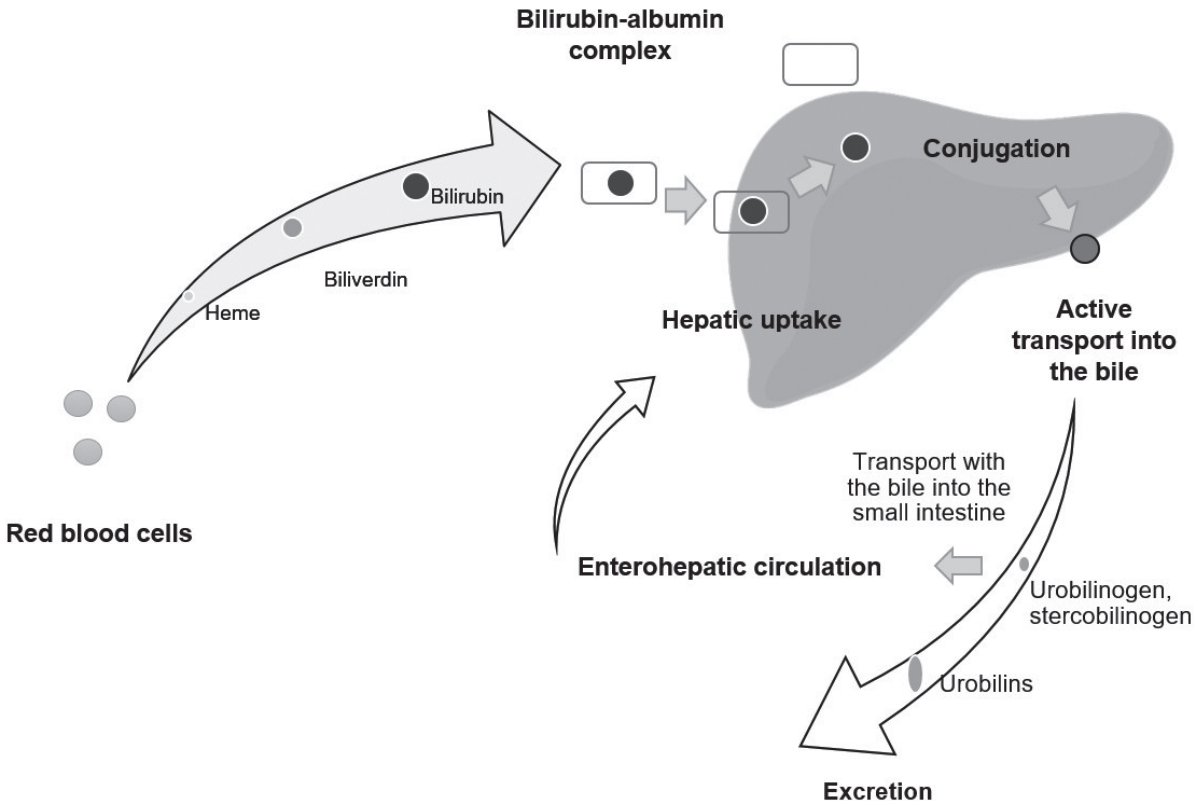

Fig. 2: Bilirubin transport. Bilirubin is enzymatically formed from heme via biliverdin. In the circulation, bilirubin is bound to albumin and transported as a bilirubin-albumin complex into the liver. In the endoplasmic reticulum of hepatocytes, bilirubin is conjugated and actively transported into the bile. In the small intestine, bilirubin is degraded to urobilinogens and stercobilinogens that are further oxidized to urobilins. A portion of urobilinogen is reabsorbed via enterohepatic recycling.

Biological effects of bilirubin

Toxicity of bilirubin

Toxic effects of bilirubin are determined by the unbound Bf fraction which can easily cross cell membranes and interfere with mitochondrial respiration leading to cell lysis or disruption of mitochondrial function [12]. When the plasma levels of unconjugated BR are massively elevated and BR accumulates in the circulation, it might result in harmful effects especially for infants. The selective neurotoxicity of BR for the neonates is due to incomplete development of the blood–brain barrier which is then more permeable. To avoid a risk of neurotoxicity, phototherapy is applied to reduce the levels of BR in jaundiced newborns using light energy to change the shape and structure of BR and converting BR to molecule that can be easily excreted from the body [13].

Antioxidant effects of bilirubin

Even though there had been some evidence about protective effects of BR, it was not until 1987 when a breakthrough paper "Bilirubin is an antioxidant of possible physiological importance" by Roland Stocker et al. was published in Science, and an intensive research on this topic has started since then [14]. The physiological concentrations of BR in the organism are relatively low but its antioxidant effects might be increased by bilirubin-biliverdin redox cycle, in which is BR regenerated by biliverdin reductase [2]. BR was found to be a free radical scavenger by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical [14] and an inhibitor of superoxide production by mechanism employing the inhibition of NADPH oxidase [15]. BR is able to protect fatty acids, phospholipids and proteins against peroxidation and neuronal cultures from the oxidative stress [16]. For example, the capacity in the protection of LDL particles against oxidative damage had been considered twenty times more efficient than that of other known scavenger of free radicals, vitamin E [17]. The antioxidant effect of BR was confirmed in several clinical studies and a direct correlation between concentration of BR and total plasma antioxidant capacity was demonstrated. Moreover, low plasma BR levels have been found to be strong and independent risk factors for coronary artery diseases [18]. Clinical studies exploring the role of plasma bilirubin concentrations and diseases associated with oxidative stress and chronic inflammation revealed negative association between plasma BR level and the risk factors for atherosclerosis, cancer or multiple sclerosis [19, 20]. For example, in case of atherosclerosis, increased concentration of BR by 1 μM is associated with reduced risk of developing this disease by 6 % in men [21].

Anti-inflammatory effects of bilirubin

Inflammation can be characterized as the body's defensive response to injury, tissue damage or infection, and involves many systemic and metabolic changes. The immediate reaction called an acute phase response takes place twenty-four to forty-eight hours after the primary insult. This first response includes initial tissue injury resulting in a cytokine release (mediators of inflammation) and receptor binding with subsequent activation of signaling cascades followed by the synthesis and release of acute phase proteins. Cytokines belong to a group of small soluble peptides (< 40 k-Da) and they are synthesized and released from various cell types during inflammation. The pro-inflammatory cytokines include tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon (IFN γ), which can further stimulate the synthesis of other cytokines [22], such as anti-inflammatory cytokine IL-10. Secretion of these cytokines is under the control of nuclear factor kappa B (NF-κB). NF-κB is a transcription factor and regulator of numerous genes involved in the immune and inflammatory responses. In resting cells, NF-κB is located in the cytoplasm in inactive state bound to the inhibitor IκB which prevents its translocation into the nucleus. When IκB is dissociated, NF-κB is free to move to the nucleus and activates specific genes. Dissociation of IκB requires phosphorylation of this factor. Phosphorylated IκB is then ubiquitinated and selectively degraded by the proteasome complex [23]. It was found that higher concentration of BR could suppress some pro-inflammatory cytokines (IL-2, TNF-α, IFN) [24]. It was hypothesized that BR could prevent translocation of NF-κB to the nucleus probably by inhibition of phosphorylation IκB [24]. This effect could be also induced by the heme-oxygenase 1, responsible for bilirubin production [25].

First clinical evidence of anti-inflammatory effects of bilirubin was observed in patients with rheumatoid arthritis over 75 years ago. These patients showed a remission of symptoms after developing jaundice from liver disease [26]. Moreover, the negative association between concentration of BR and incidence of chronic inflammatory diseases has been found in individuals with Gilbert syndrome (who have chronically mildly elevated BR in serum) [27]. Another example is systemic lupus erythematosus, an inflammatory disease associated with an increased oxidative stress and characterized by complement system aberrations, defects in antigen presentation and an abnormal adaptive immune response. Male as well as female patients with systemic lupus erythematosus had almost fifty percent lower serum total BR levels than healthy controls [28]. Those patients with the lowest concentrations of serum BR were more likely to have multi organ disease secondary to lupus [19]. A large epidemiologic study confirmed that higher total serum BR levels were associated with a reduced risk of rheumatoid arthritis in humans [24]. However, the clear role of BR in inflammation processes has not been fully established yet.

Immunomodulatory effects of bilirubin

Increasing evidence suggests that BR could possess also immunomodulatory properties. The first evidence about effects of BR on the functions of cells of the immune system was published almost thirty years ago. It was found that hyperbilirubinemia exerted a

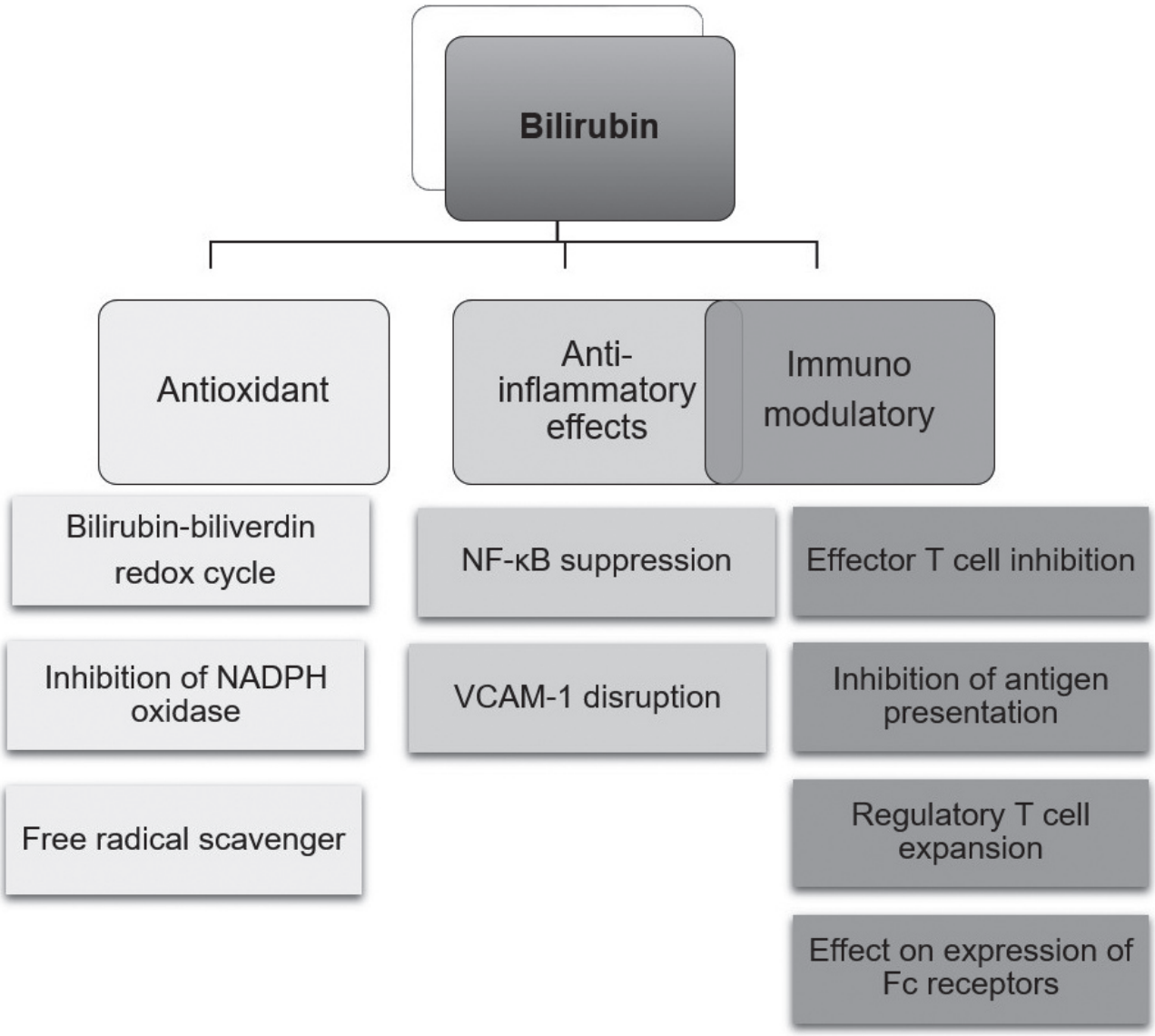

Fig. 3: The overview of protective effects of bilirubin.

suppressive effect on antibody formation in newborn infants which supported the hypothesis that BR plays a role in the development of the immune system [30]. During last years, it was discovered that BR significantly inhibited antigen-specific and polyclonal T cell responses, for example high levels of BR induced apoptosis in reactive CD4 T cells and inhibited cell-surface expression of MHC II class molecules on antigen-presenting cells [24]. Another study showed a possible influence of BR on the expression of Fc receptors in macrophages [31]. Recent investigations suggest that BR can induce expansion of regulatory T cells (Treg) [32] which are involved in the prevention of autoimmune diseases and in the suppression of the immune system tolerance probably by the induction of regulatory gene Foxp3 [33]. It was also published that bilirubin prevents formation of atherosclerotic plaque by inhibiting monocyte migration across the vascular endothelium through disruption of vascular cell adhesion molecule-1 (VCAM-1) signaling *in vitro* [34] and ameliorates VCAM-1-mediated airway inflammation *in vivo* [35]. These various protective effects of BR are summarized in Fig. 3.

Conclusion

Bilirubin is an important molecule with multiple biological functions. While extremely high serum concentrations of unconjugated bilirubin can cause severe neurotoxicity in humans, mildly elevated bilirubin levels exert protective action. The mechanism of the protective effects of bilirubin has not been fully elucidated including the clear mechanism of immunomodulatory and anti-inflammatory activities, so further research is needed.

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