Enterohepatic circulation of bilirubin

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List of publications

1. Vitek L., Muchova L., Zelenka J., Zadinova M., Malina J. The Effect of Zinc Salts on Serum Bilirubin Levels in Hyperbilirubinemic Rats. J. Pediatr. Gastroenterol. Nutr. 2005;40:135–140.

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10. Zelenka J., Lenicek M., Muchova L., Jirsa M., Kudla M., Balaz P., Zadinova M., Ostrow JD., Wong RJ., Vitek L. Highly sensitive method for quantitative determination of bilirubin in biological fluids and tissues. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 2008;867:37-42.

Abbreviation:

BP:	Bile pigments
BS:	Bile salts
BVR:	Biliverdin reductase
CO:	Carbon monoxide
EHC:	Enterohepatic circulation
ESC:	Enterosystemic circulation
HO:	Heme oxygenase
UCB:	Unconjugated bilirubin
UGT1A1:	Bilirubin UDP-glucuronosyltransferase

Introduction

Metabolism of bilirubin and other bile pigments

Production of bilirubin

Bile pigments (BP), also known as bilins or bilanes, belong to the group of substances descending from degradation of heme and bearing common structural motif of linear tetrapyrrole. A number of BP with various physiological functions was described to be produced by animals, plants and procaryotic microoorganisms [1]. The range of BP functions is rather broad and generally connected with light absorption and/or electron transfer. For example, biliverdin serves as a pigment causing green color of reptiles and amhibians, phycocyanobilin and phycourobilin are part of a light harvesting complex of blue-green algae and bilirubin is a potent antioxidant protecting mammalian cells from lipoperoxidation [2].

Human heme degradation pathway is opened by the microsomal enzyme heme oxygenase (HO, 1.14.99.3) which oxidatively cleaves heme moiety producing biliverdin, carbon monoxide (CO) and ferrous cation (Fig. 1). HO occurs in two isoforms, HO-1 and HO-2. HO-1, belonging to heat shock proteins (HSP32), is highly inducible by various factors causing oxidative stress, e.g. inflammation, UV irradiation, heavy metals or hydrogen peroxide. In contrast, HO-2 is expressed constitutively [3]. Expression of HO-1 has important antioxidative, antiinflammatory and antiapoptotic effects mediated by hemin sequestration, production of CO and BP [3] as well as by direct action of HO-1 as a transcription factor [4]. While CO is a well documented signalling molecule [5], data on the role of BP in signal transduction are rather limited [6].

Biliverdin, the blue-green polar substance, is present only in trace amounts in the human body. It is readily reduced by enzyme biliverdin reductase (BVR, 1.3.1.24) forming major mammalian bile pigment bilirubin (Fig.1). BVR is a microsomal and cell surface enzyme playing an important role also in cellular oxidative stress signalling. Beside its biliverdin reducing role, it is also dual specificity (Ser/Thr/Tyr) kinase with proteinkinase-C activity. Moreover, after cleavage and nuclear translocation, it serves also as a transcription factor for various antioxidant genes [7].

Unconjugated bilirubin (UCB) is the orange substance sensitive to light and oxygen. Interestingly, its physico-chemical properties are strongly pH dependent. While UCB is well soluble in alkaline aqueous solutions, it becomes very hydrophobic below pH 8 and readily partitions into some nonpolar solvents. This is caused by hydrogen bonds formed in diacid species of UCB creating polar core of its molecule while nonpolar structures remain exposed on its surface (Fig. 2). Importantly, most of UCB is in a nonpolar form under physiological conditions and is bound to proteins and membranes [8]. In addition, UCB physiologically possesses important antioxidant and antiinflammatory properties [9]. However, it could be neurotoxic under pathological conditions [10]. Most of UCB is produced as a result of continuous degradation of hemoproteins including hemoglobin from senescent red blood cells in spleen and is transported from peripheral tissues to the liver bound to serum albumin. Hepatocytes efficiently uptake UCB and conjugate it with glucuronic acid using an enzyme bilirubin UDP-glucuronosyltransferase (UGT1A1, 2.4.1.17). Bilirubin monoglucuronoside or bisglucuronoside or other minor conjugates are then secreted into bile [11].

Fig. 1: Heme degradation pathway in human body.

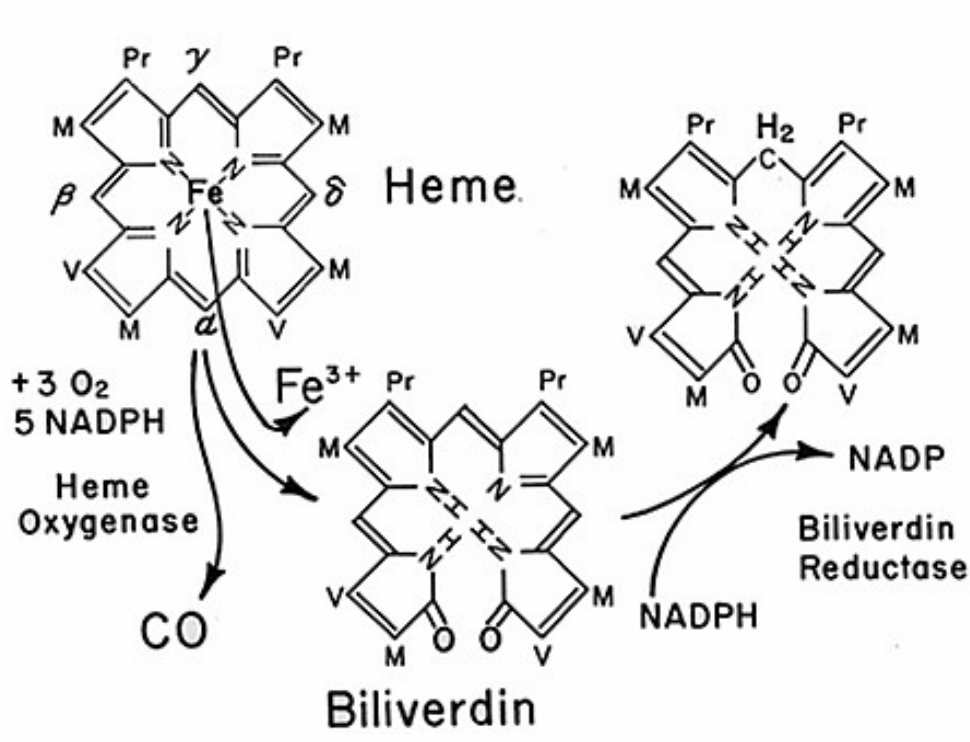
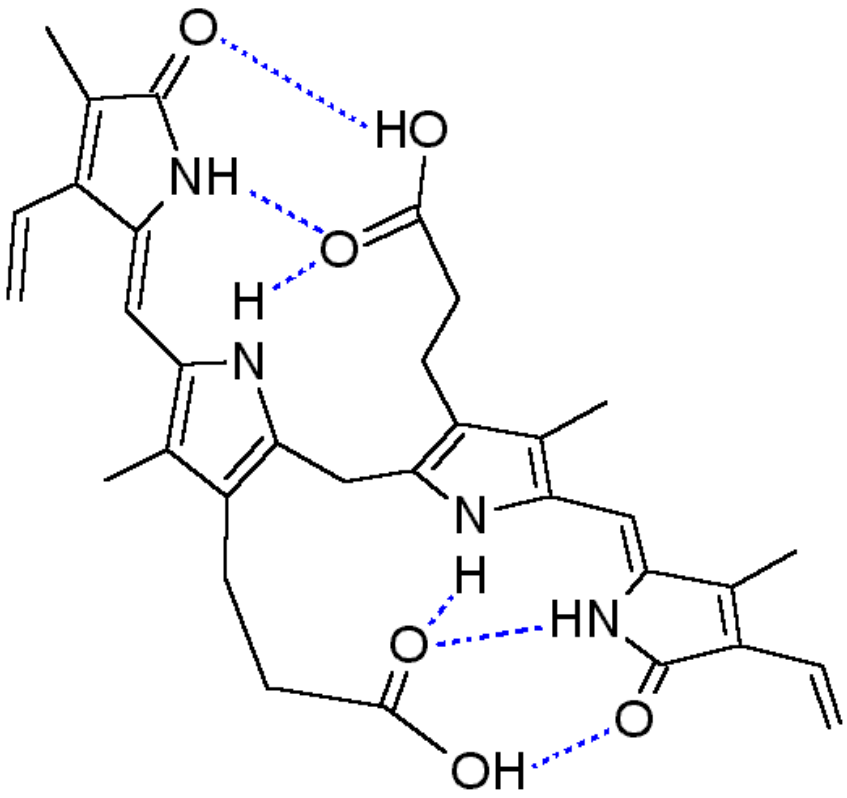


Fig. 2: Structure of intramolecular hydrogen bonds in the bilirubin molecule



UGT1A1 is a member of a large family of glucuronosyltransferases, which catalyses phase II detoxication of various xenobiotics as well as endogenous nonpolar substances including hormones and drugs. Certain polymorphisms in the promoter of *UGT1A1* gene may cause decreased UCB conjugation which in turn leads to manifestation of benign unconjugated hyperbilirubinemia known as a Gilbert syndrome [12]. Prevalence of Gilbert's phenotype in the Caucasian population is 5 – 7% and it was proved that these subjects are protected against various inflammatory and oxidative stress-related diseases (see below) [9,12]. On the other hand, insufficient activity or complete lack of the enzyme function leads to severe unconjugated hyperbilirubinemia, and under conditions of compromised blood-brain barrier, such as during neonatal period, it may result in severe neurological damage called kernicterus or bilirubin encephalopathy [13]. This can happen in neonates with severe neonatal jaundice or in patients with inherited metabolic disorder of UGT1A1 known as Crigler-Najjar syndrome. Animal model for UGT1A1 deficiency is represented by Gunn rats, the strain of Wistar rats with spontaneous genetic deletion [14].

Physiological role of UCB

For decades, bilirubin was considered to be a waste product of heme degradation. Interestingly, most of vertebrates produce only biliverdin which is polar, nontoxic and easily secretable without conjugation. In contrast, mammalian species quantitatively reduce biliverdin to potentially dangerous bilirubin. It was hypothesized that only bilirubin, but not biliverdin could cross the placental barrier by spontaneous diffusion due to its relative hydrophobicity thus preventing accumulation of BP in the fetus. However, further investigations have shown that the fetus can produce more polar isomer bilirubin IXβ [15]. Moreover, multidrug resistance-associated protein-1 (MRP1, ABCC1) was found to actively transport UCB through the placental barrier thus compromising this hypothesis [16].

A breakthrough came when antioxidant activity of bilirubin was explored [17]. It was found that UCB as well as conjugated bilirubin are potent antioxidants *in vitro* and protect serum albumin as well as LDL particles against various free redical species [18]. In addition, a number of clinical studies have shown that subjects with Gilbert syndrome are protected from development of atherosclerosis [19], colorectal cancer [20] and number of other diseases and injuries accompanied with oxidative stress and chronic inflammation [9]. Although cellular concentrations of UCB are by several orders of magnitude lower than that of other antioxidants (e.g. glutathione, free amino acids), bilirubin can protect cells against 10,000x higher concentration of hydrogen peroxide [21]. This effect is most likely due to a concentration of UCB in membranes where it efficiently breaks the oxidative chain reactions oxidizing itself to polar biliverdin which is subsequently reduced back to UCB by ubiquitous BVR [21]. Organisms like mammals with their very intensive metabolism need a strong antioxidant protection and production of UCB could be thus beneficial although energetically more expensive and potentially dangerous.

Intestinal metabolism of bile pigments

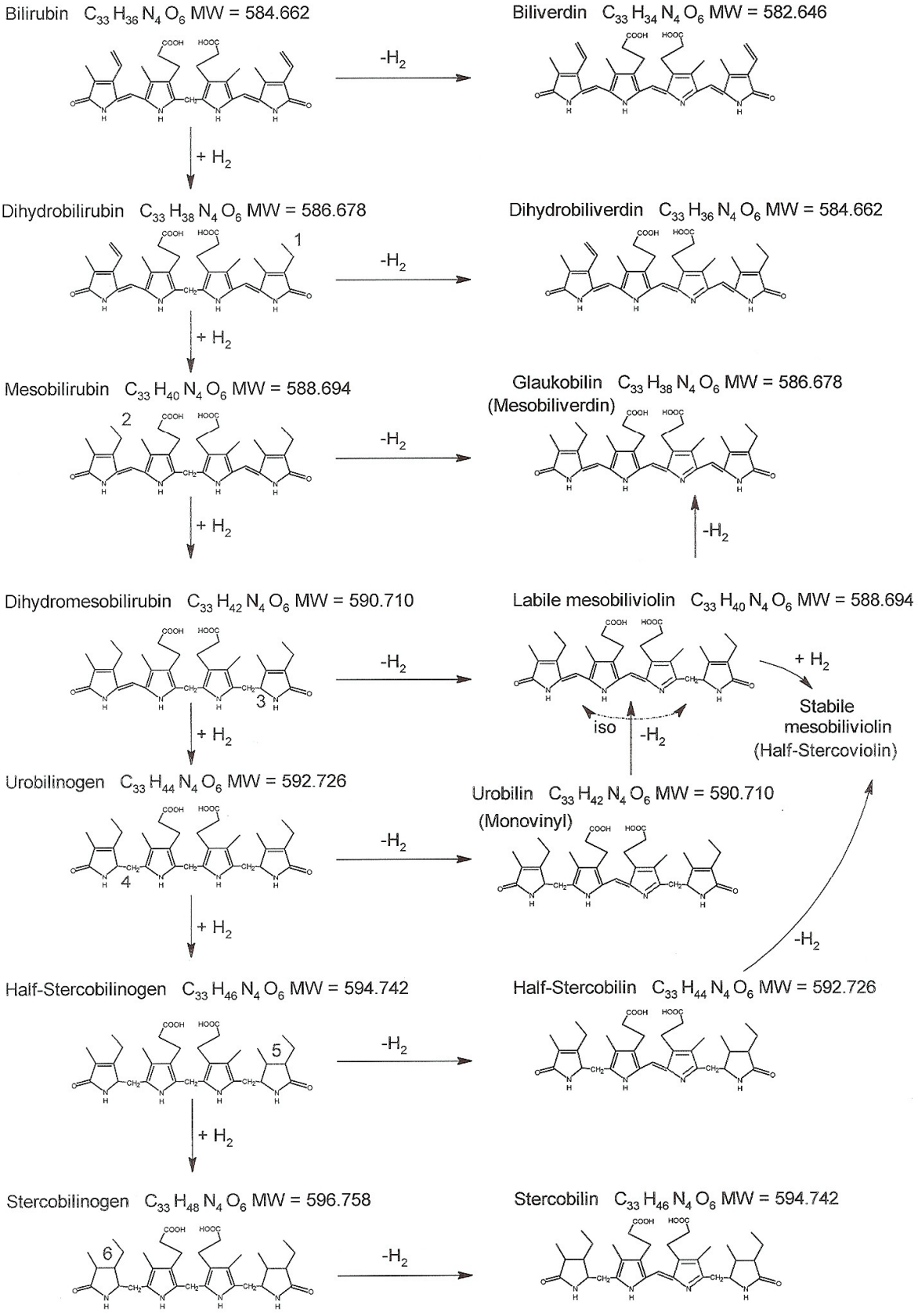
Conjugated bilirubin is secreted into bile via active transporter called multidrug resistance-associated protein-2 (cMOAT, MRP2, ABCC2) which serves as a canalicular exporter for various and endogenous compounds. Defect of transporter function called Dubin-Johnson syndrome or restricted bile flow (cholestasis) result in

conjugated hyperbilirubinemia [22]. Under these conditions, polar conjugated bilirubin is effluxed by basolateral transporter multidrug resistance-associated protein-3 (MRP3, ABCC3) into blood and excreted into urine [23].

During its passage through the small intestine and colon, most of bilirubin is deconjugated. Deconjugation is catalyzed by β-glucuronidase (3.2.1.31), an enzyme produced in large amounts by colonic microflora [24] but present also in bile canaliculi [25] or coming from milk during breast feeding [26]. Part of the intestinal UCB is reabsorbed and transported via portal vein to the liver [27] and certain part also to the systemic circulation [28]. This process, called enterohepatic and enterosystemic circulation (EHC, ESC) is influenced by the ratio between bilirubin deconjugation and transformation to other compounds and by the relative solubility of UCB in the intestinal content [29].

However, under physiological conditions, the vast majority of UCB remains in the intestine and is hydrogenated by the intestinal microflora (Fig. 3). Major products of reduction are urobilinogen and stercobilinogen - the bilirubin derivatives with eight and twelve additional hydrogens, respectively. These polar colorless substances are unstable and could be easily oxidated to orange-yellow urobilin and stercobilin which determine typical color of feces. This group of pigments is collectively called urobilinoids [30]. Part of UCB is also transformed by nonspecific reactions to poorly defined mono-, di- and oligopyrroles. In contrast, reduction to urobilinoids is highly specific and quantitative. As much as 30 - 60% of excreted bilirubin is converted to urobilinoids [30]. Reduction activity was generally attributed to the gender *Clostridium* [31]. Moreover, particular strains of *C. perfringens* and *C. difficile* capable to reduce bilirubin were identified in our laboratory [32]. The adaptive value of this metabolic pathway remains to be clarified. It could be hypothesized that this reduction serves as an anaerobic respiration or as an detoxication mechanism against nonpolar UCB which can impair membrane functions.

Fig. 3: Metabolism of urobilinoids. Sequential reduction of UCB to urobilinogen and stercobilinogen and appropriate oxidation to corresponding bilins.



EHC of bilirubin under physiological and pathological conditions

EHC as a mechanism for fasting hyperbilirubinemia

Fasting leads to bilirubin elevation in humans as well as in rodent models [33]. Rise of serum UCB is mild in normal individuals but could be more intense in Gilbert syndrome subjects. Due to this fact, it used to serve as a possible diagnostic tool [34]. Recently, EHC and ESC of bilirubin were found to be the most likely mechanisms for fasting-induced hyperbilirubinemia [35]. Fasting related increase in gastrointestinal transition time causes increased retention of UCB in the intestine. Intestinal UCB is then more available for reabsorption into portal circulation. Since only 1/3 of UCB is resecreted into bile during the first pass [28], substantially high amount of UCB escapes into systemic circulation. Although biliary bilirubin output is also enhanced, low intestinal motility with increased UCB reabsorption causes more intensive reabsorption resulting in elevation of serum UCB. Gilbert syndrome subjects are more prone to fasting hyperbilirubinemia because their hepatic UCB conjugation is less efficient thus unable to compensate increased hepatic load of UCB [35].

EHC during neonatal jaundice

Neonatal jaundice is an unconjugated hyperbilirubinemia resulted predominantly from high rate of hemoglobin degradation and insufficient conjugating capacity of the newborn liver. It is believed that elevated UCB levels provide a substantial protection against oxidative stress resulting from transition into relatively hyperoxic environment after delivery [36]. However, 8 - 20% of healthy, full term newborn infant develop potentially dangerous jaundice with peak serum bilirubin levels above 220 μmol/L. In the newborn period, blood brain barrier does not protect central nervous system completely and very high UCB levels can injure neurones and cause significant neurological damage, which can lead to lifelong sequele or even death [13]. Another important factor contributing to the pathogenesis of neonatal jaundice is increased EHC and ESC of bilirubin. This is caused by several independent conditions. Most importantly, neonatal intestine is sterile after birth and colonised slowly with bilirubin reducing microflora which would normally decrease

UCB availability by its transformation to urobilinoids [32]. In addition, maternal milk contains high activity of β-glucuronidase which readily deconjugate bilirubin [26]. Thus, colonic UCB levels are much higher than in adults forming gradient between intestinal lumen and portal circulation which enhances EHC and ESC. Nowadays, maternal milk restriction is recommended as a treatment of prolonged neonatal breast milk jaundice. However, direct hypobilirubinemic influence of UCB reducing activity associated with colonic microflora has never been investigated although it could lead to development of safe treatment for neonatal jaundice.

EHC and pigment gallstones

Pigment gallstone disease is a relatively common biliary tract disorder with an estimated 20-25% clinical prevalence among patients undergoing cholecystectomy in the United States. It was found that pigment gallstones are formed mainly by calcium bilirubinate [29]. The development of pigment concrement requires presence of high concentration of UCB in a bile duct. EHC could significantly increase biliary bilirubin levels with increased proportion of bilirubin monoglucuronoside instead of bilirubin bisglucuronoside due to saturation of conjugation enzymes. This phenomenon is particularly enhanced in Gilbert syndrome subjects and in patients with bile salt (BS) malabsoption. In Gilbert syndrome subjects, UCB conjugation is impaired and significant amount of bilirubin monoglucuronoside is secreted into bile. This species tends more easily to deconjugate increasing UCB concentration in the bile duct. Higher intestinal UCB levels favor EHC leading to further rise in bilirubin monoglucuronoside and UCB in bile. UCB due to its natural insolubility in aqueous solution could precipitate in the form of calcium salts thus forming pigment gallstones [29]. BS malabsorption occurs when distal ileum is compromised due to inflammation or surgical resection. This causes increased leak of BS into colon. While intestinal UCB is normally poorly soluble in the intestinal content, BS increase its solubility and enhance its EHC. Higher biliary bilirubin output also increases proportion of biliary UCB thus leading to gallstone formation [37].

Intestinal excretion of UCB during extreme hyperbilirubinemia

In contrast to previously discussed intestinal reabsorption of UCB, under conditions of extreme unconjugated hyperbilirubinemia could be UCB also directly secreted through the intestinal wall. In case of complete lack of UCB conjugation activity, bilirubin is not secreted into bile or urine and is accumulated in the body reaching very high levels. Under these conditions, steep gradient between serum and intestinal UCB emerge leading to direct excretion of bilirubin by the intestinal wall. This process takes place along the whole lenght of intestine and is probably directed by passive diffusion of nonpolar UCB [38]. This interesting phenomenon is the main pathway of bilirubin elimination in patients with Crigler-Najjar syndrome as well as in Gunn rats which serve as a model for investigation of neonatal jaundice.

Regulation of EHC using UCB binding agents

Level of free intestinal UCB is the main driving force for EHC. UCB is generally poorly soluble in aqueous solutions and is easily adsorbed to other biomolecules [8]. BS are the only physiologically relevant agents increasing its intestinal solubility. Water soluble UCB-BS complex highly enhances mobility of UCB thus triggering its EHC and ESC. Especially during BS leakage into colon (malabsorption or oral supplementation of BS), EHC could significantly influence biliary bilirubin output [37].

On the other hand, there are numerous agents causing intestinal UCB precipitation or strong adsorption thus inhibiting EHC and ESC. Adsorbents like agar [39], active charcoal [40] and cholestyramine [41] or precipitation agents like calcium phosphate [42] and zinc sulphate [43] were succesfully tested in animal models and pilot trials with Crigler-Najjar patients. However, therapeutic value of these substances for treatment of neonatal jaundice or pigment gallstone formation is discutable due to possible adverse effects and inconsistent results from clinical studies [42].

Generally, regulation of EHC and ESC of bilirubin could be an important therapy especially for treatment of neonatal jaundice, however, it requires further intensive investigation.

Aims

Inhibition of EHC of bilirubin by agents decreasing its intestinal solubility is a promising approach in treatment of neonatal jaundice. However, adsorbents and precipitation agents used so far were not clinically applicable. In our first paper entitled **„The effect of zinc salts on serum bilirubin levels in hyperbilirubinemic rats“**, we aimed to investigate hypobilirubinemic effect of zinc methacrylate - the unabsorbable adsoption/precipitation agent.

Our knowledge on the role of intestinal microflora in bilirubin EHC is not complete. Therefore, we isolated several strains of bacteria from neonatal stool and characterized their bilirubin reducing activity. The aim of our next paper entitled **„The impact of intestinal microflora on serum bilirubin levels“** was to investigate their influence on serum UCB levels in Gunn rats.

The intestinal metabolic pathway of BP is known only in context of the whole intestine. Metabolism of particular strain of bilirubin reducing bacteria has never been investigated. The aim of our third study entitled **„Identification of bilirubin reduction products formed by *Clostridium perfringens* isolated from human neonatal fecal flora“** was to investigate BP metabolism in the sole strain of *Clostridium perfringens* with high biliruin reducing activity.

Metabolism of bilirubin under physiological and pathological conditions was investigated only indirectly using determination of bilirubin species in serum, urine or bile. However, knowledge on the tissue and cellular UCB levels are principial for our further understanding of bilirubin metabolism. Unfortunately, UCB is unstable, has high affinity to biomolecules and tissue UCB levels are very low. For these reasons, there has been no analytical procedure for sensitive determination of bilirubin in the complex biological matrices. In our final paper entitled **„Highly sensitive method for quantitative determination of bilirubin in biological fluids and tissues“**, we aimed to develop such a method.

Vitek L., Muchova L., Zelenka J., Zadinova M., Malina J.
The Effect of Zinc Salts on Serum Bilirubin Levels in Hyperbilirubinemic Rats.
J. Pediatr. Gastroenterol. Nutr. 2005;40:135–140.

Vitek L., Zelenka J., Zadinova M., Malina J.

The impact of intestinal microflora on serum bilirubin levels.

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Discussion

In the presented papers, important aspects of EHC of bilirubin were characterized in rodent models. Generally, novel potential approaches for therapy of neonatal jaundice have been explored in the first two publications while the other two characterized the BP metabolism in the bacterial cells and mammalian tissues, respectively.

In the first publication entitled „**The effect of zinc salts on serum bilirubin levels in hyperbilirubinemic rats**“, an important decrease in serum bilirubin levels was found in response to feeding animals with insoluble UCB binding agent. In contrast with previously tested zinc and calcium salts [42,43], zinc methacrylate not only decreased hyperbilirubinemia but also had no effect on inorganic ions homeostasis. However, the relevance of zinc methacrylate for future treatment of severe unconjugated hyperbilirubinemia is discutable. Although we found no pathologic changes in the rat intestine at the end of the experiment, methacrylate should be considered as a potentially toxic agent. However, the important conclusion from this study is, that intestinal passage of unabsorbable and inert material with high ability to specifically adsorb UCB (e.g. using divalent cationts) can significantly decrease level of unconjugated hyperbilirubinemia due to its ability to restrict EHC and ESC of bilirubin. Future use of biocompatible polymers without any harmful interaction with organism could lead to development of a new therapy for Crigler-Najjar patients or babies with neonatal hyperbilirubinemia. Such approach could be an alternative to current therapies used for neonatal jaundice such as phototherapy and exchange transfusions [13].

Another approach for restriction of EHC and ESC of bilirubin is the enhancement of bilirubin reduction. This is highly suitable especially for treatment of neonatal jaundice in newborns lacking effective intestinal microbial ecosystem [32]. In the second experimental paper entitled „**The impact of intestinal microflora on serum bilirubin levels**“, we proved that eradication of bilirubin reducing microflora had led to increase in serum bilirubin levels in Gunn rats, while recolonization with single strain possessing UCB reducing capacity resulted in an important drop of hyperbilirubinemia. Thus, treatment of newborns with probiotic bacteria capable to reduce intestinal bilirubin could significantly decrease the level of hyperbilirubinemia.

However, bilirubin reducing capacity was found to be associated with potentially pathogenic strains of *Clostridium perfringens* and *C. difficile* whose application as probiotic agents for newborns is highly unlikely. The solution might be the preparation of a safe transgenic bacterial strain with enhanced bilirubin reducing activity. For this reason, further characterization of metabolic pathway leading to urobilinoids is highly needed. It could be hypothesized, that bilirubin reduction is either nonspecific action of some reductase, detoxication mechanism against potentially toxic UCB or highly specific procedure serving as an anaerobic respiration. In the latter case, a specific enzyme could develop from proteins, which synthesize various bilins in blue-green algae [2]. Structure of these bilins and urobilinoids is similar. Thus, real nature of bilirubin reducing metabolic pathway remains to be clarified.

To characterize BP metabolism in particular microorganism, production of urobilinoids was characterized in the strain of *C. perfringens* isolated from neonatal stool. In the third publication entitled „**Identification of bilirubin reduction products formed by *Clostridium perfringens* isolated from human neonatal fecal flora**“, unconjugated urobilinogen was found as a main physiological product of UCB reduction in this bacteria. Moreover, production of urobilinoids was also possible from UCB derivatives mesobilirubin, bilirubin dimethylester, bilirubin bisglucuronoside and much less effective also from bilirubin ditaurate and bilirubin diethylester. Bilirubin bisglucuronoside was found to be deconjugated prior to reduction. If the partially purified enzyme from bacteria was used as a catalyzator, bilirubin bisglucuronoside was not reduced at all. It means that substrate specificity of possible enzyme is rather broad but reduction is not nonspecific. Moreover, whole procedure is quite complex and requires orchestral action of reducing enzymes together with β-glucuronidase which hydrolyse bilirubin glucuronosides prior to reduction and probably also with some membrane carrier transporting bilirubin across the cellular membrane. This is suggested by the observation, that polar derivative bilirubin ditaurate, which cannot cross membrane spontaneously, is reduced by intact bacteria without deconjugation (unpublished observation). Due to intracellular localization and NADH dependency of bilirubin-reducing enzyme, treatment of neonatal jaundice with simple enzyme preparation is unlikely. Thus, further characterization, isolation and finally identification of DNA sequence encoding gene for bilirubin reductase is needed for potential preparation of transgenic probiotic bacteria bearing bilirubin reducing activity.

Not only the end of BP metabolic pathway but also its beginning in mammalian cells and tissues is still not fully understood. This is also due to difficulties in BP analysis. Since UCB is highly sensitive to oxidation and possesses high affinity to biomolecules [8], there has been no reliable method for determination of low physiological UCB levels in cells and tissues. In our final publication entitled „**Highly sensitive method for quantitative determination of bilirubin in biological fluids and tissues**“, we developed such a highly sensitive and precise method and used it to determine bilirubin levels in tissues from normobilirubinemic Wistar and hyperbilirubinemic Gunn rats. Tissue UCB levels were found to span over 4 orders of magnitude from 40 pmol/g in fat and brain of Wistar rat to 80 nmol/g in liver of Gunn rats. Such an extreme variability suggests that there has to be an active regulation of UCB concentrations in particular cells. Thus, the novel method is a suitable tool also for investigation of UCB cytotoxicity, detoxication mechanisms and active transport together with correlation of HO-1 activity with production of bilirubin, study of changes in UCB levels in response to oxidative stress and possible antiinflammatory and cytostatic function of UCB. The knowledge of these processes together with deeper understanding on bilirubin EHC and intestinal metabolism will help to treat neonatal jaundice and, on the other hand, regulate UCB levels to enhance its beneficial antioxidant and antiinflammatory action.

Summary

Bilirubin is a main physiological product of heme degradation possessing important antioxidant and antiinflammatory properties. On the other hand, it could be neurotoxic during severe unconjugated hyperbilirubinemia combined with insufficiency of blood-brain barrier (neonatal jaundice). It is secreted from the body via bile and is further metabolized in the intestine. Part of the substance is reduced to urobilinoids, part is adsorbed to the intestinal content and some part could be reabsorbed back to the systemic circulation. This enterohepatically and enterosystemically circulating fraction varies in size depending on the rate of bilirubin secretion, solubility in the intestine and intensity of its intestinal metabolism. Under specific circumstances, EHC and ESC may significantly increase serum and bile bilirubin levels and influence physiological as well as pathological processes occuring in the body. Among the most important is the protective elevation of UCB levels in Gilbert syndrome subjects and dangerous increase in severity of neonatal jaundice.

In the presented thesis, the mechanisms affecting EHC and ESC of bilirubin and tools for further research in BP metabolism were investigated. The solubility of intestinal UCB is strongly decreased by addition of divalent cations. However, such approach to decrease EHC of bilirubin could be significantly harmful to metabolism of inorganic ions. The solution might be the use of insoluble matrices containing exposed divalent cations. Our study proved that feeding of hyperbilirubinemic Gunn rats with zinc methacrylate led to an important decrease in serum bilirubin levels without affection of zinc metabolism and pathologic changes in the intestine. Another important factor decreasing EHC is hydrogenation of intestinal UCB by intestinal bacteria belonging to the genus *Clostridium*. We proved that eradication of intestinal *Clostridia* of Gunn rats led to significant increase in hyperbilirubinemia while recolonization with a sole strain of *C. perfringens* capable of reducing bilirubin partially restored the bilirubin homeostasis. Bilirubin metabolism of this strain was further characterized using chromatographic methods. Urobilinogen was found to be a main product of hydrogenation of a number of substrates including mesobilirubin, bilirubin dimethylester, bilirubin ditaurate but not bilirubin bisglucuronoside. The investigation of cellular and tissue bilirubin metabolism is important for understanding of EHC and ESC of bilirubin as well as its antioxidant and toxic properties. However,

due to its low cellular levels and high instability, valid analytical method for tissue bilirubin determination was unavailable. Therefore, we developed and validated a highly sensitive and precise HPLC assay for quantification of bilirubin in cells and tissues under normobilirubinemic as well as hyperbilirubinemic conditions.

In conclusion, we found that regulation of EHC and ESC of bilirubin is a promising approach to control hyperbilirubinemia. Furthermore, we also partially characterized a metabolic pathway of intestinal bilirubin degradation. Finally, we developed a novel analytical method for further investigation of bilirubin metabolism.

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