DIETARY COMPOUNDS THAT MODIFY BILIRUBIN LEVELS

Rosa Pereira[a]*, Sandrine Monteiro[a]*, Carina Rodrigues[a,b]*, Josiana Vaz[a,c] and Isabel C.F.R. Ferreira[c]

[a] School of Health, Polytechnic Institute of Bragança, Bragança, Avenida D. Afonso V - 5300-121 Bragança, Portugal.

[b] Research Unit on Applied Molecular Biosciences - REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal.

[c]Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

INTRODUCTION

Bilirubin (BL) is a yellow-orange pigment resulting from the catabolism of hemeproteins. This tetrapirrolic metabolite belongs to one of the most conserved superfamily of molecules in living, organisms. The BL has been subject of study for more than three centuries. In clinical diagnosis, BL is a marker of liver function and used to monitoring hematological diseases. The enzyme that catalyzes BL conjugation is the uridine diphosphate glucuronyl transferase 1A1 (UGT1A1). Unconjugated BL (UCB) is lipid soluble molecule but after conjugation (with one or two molecules of glucuronic acid) becomes water soluble (conjugated bilirubin: CB), allowing its excretion via the bile canaliculi. The most prevalent metabolic disorder in the Caucasian population is Gilbert's syndrome (GS). It´s a benign condition, characterized by moderate hyperbilirubinemia in the absence of hemolysis or liver dysfunction. The common variant associated with this syndrome is the TA duplication at position c.-41_-40dupTA (variant UGT1A1*28 or A(TA)$_7$TAA allele) located in the promoter region of the UGT1A1 gene. The presence of hyperbilirubinemia, associated with GS, can lead to the worsening of clinical symptoms of individuals with chronic hemolytic diseases.

At high concentrations, as described in children with Crigler-Najjar syndrome type I (SCN-I) or type II (SCN-II), BL can be extremely toxic. However, in the past 20 years, the possible antioxidant, anti-inflammatory and anti-carcinogenic properties, observed in the presence of mild elevated SBL, have been motivated researchers to conduct numerous epidemiological and experimental studies to clarify the mechanisms involved in its potential protective effect.

Observation of in vitro and in vivo studies suggest that certain dietary compounds may increase or decrease BL levels. It is well known that drugs and other substances that can compete with BL for glucuronidation also contribute to the raising of SBL. Some herbal extracts can even exert inhibitory effects of UGT1A1 activity and thereby increase BL levels. On the other hand, there are dietary components that increase enzymatic activity of UGT1A1, such as citrus fruit and some constituents of such Cruciferous vegetables (eg. cabbage and broccoli) by increasing the expression of UGT1A1 gene. In animal models it was demonstrated that soy protein and soy isoflavones enhance hepatic UGT activity.

BRIEF DESCRIPTION OF BILIRUBIN METABOLISM

In human plasma there are 4 main forms of circulating BL: unconjugated bilirubin (UCB), also known as α-bilirubin or indirect bilirubin (IB); monoconjugate bilirubin (bilirubin-β) or monoglucorunide (MGB) or conjugated bilirubin (CB); diconjugated bilirubin (bilirubin γ) or diglucorunide (DGB) or direct bilirubin, also known as hepatic growth factor covalently bound to albumin, irreversibly. Another BL fraction to consider is the free BL that is not bound to albumin. BL is transported in plasma bound to Alb with a high affinity bond to the Alb primary binding site. The free BL correlates better with BL toxicity than any other fraction. The main source of BL is the heme group of hemoglobin from the destruction of senescent erythrocytes, which contributes around 80-85% of total production. The remaining 15 to 20% of BL production results from the tournover of other liver hemeproteins such as myoglobin, catalase and cytochrome. A small proportion (1-5%) results from the premature destruction of premature erythrocytes in bone marrow or spleen. The heme catabolism, resulting in BL production occurs within macrophages of the spleen, bone marrow and in Kupffer cells. After the heme breakdown BL is released into the plasma. In this mechanism, the ring of ferroprotoporfirina IX heme group, the prosthetic group of proteins such as hemoglobin, myoglobin and cytochrome P-450, suffers the catalytic action of heme oxygenase (HO-1). This enzyme consumes three molecules of oxygen and requires a reducing agent, nicotinamide adenine dinucletídeo phosphate (NADPH). The enzyme HO-1 acts at the central bridge methionine, forming biliverdin (BLV) and is located in the plasma membrane of the endoplasmic reticulum, nucleus and mitochondria. Its synthesis is induced by stimuli associated with oxidative stress, including free oxygen and bacterial lipopolysaccharide radicals by increasing the intracellular concentration of hepatic heme induced by various drugs, natural compounds, cytokines and growth factors. From the oxidation of heme it also results iron (Fe3 $^+$), carbon monoxide (CO) and BLV. The BLV is, in turn reduced to BL in a reaction that is catalyzed by biliverdin reductase (BVR), dependent on NADPH. BL at this stage, is called UCB and circulates in the blood bound to albumin.

Alb greatly enhances their solubility due to two binding sites for this molecule and also prevents it is excreted into urine (15).

The liver plays a central role in the metabolism of BL. It is responsible for their capture, storage, conjugation and excretion. The BNC circulates in plasma in a complex bound to Alb (BL- Alb) entering the hepatocyte by its surface sinusoidal (figure 1).
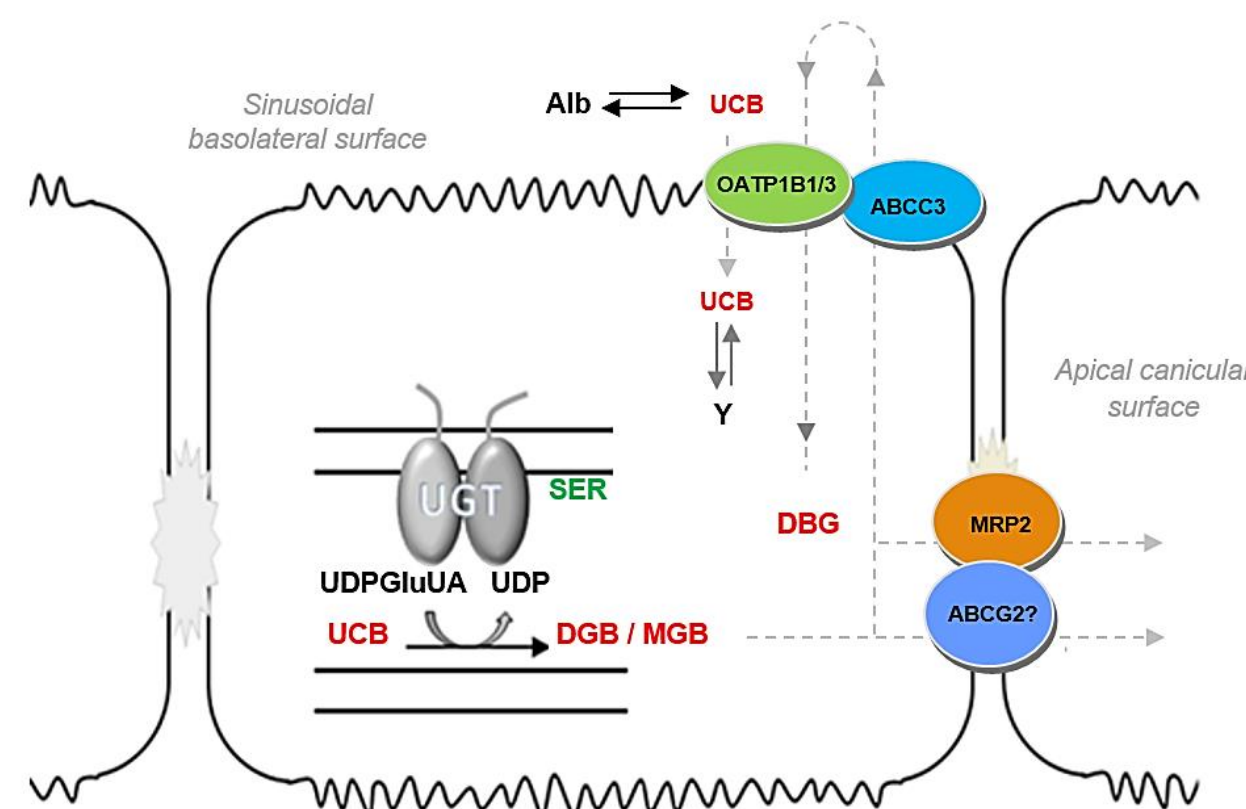

Fig. (1). Bilirubin hepatic uptake, conjugation and glucoronid transporters. SER: smooth endoplasmic reticulum; UCB: unconjugated bilirubin; Alb: albumin; MGB e DGB: mono and diglucoronid; Y: ligandin; UGT1A1: uridine 5'-diphospho-glucuronosyltransferase 1A1 (UDP-glucuronosyltransferase, UGT); UDPGlcUA: glucuronic acid residue; OATP1B1/3: organic anion transporter, 1B1 and 1B3; ABCC3: ATP-Binding Cassette transporter, (Sub-Family C, CFTR/MRP, Member 3); MRP2: Multidrug Resistance-associated Protein 2; ABCC2: ATP-Binding Cassette transporter (sub-family G member 2). The image also shows the location of UGT1A1 in the membrane of smooth endoplasmic reticulum membrane (SER); transport of unconjugated bilirubin (UCB) into hepatocyte at the sinusoidal surface by the action of the organic anion basolateral transporters OATP1B1/3 (organic anion transporter, 1B1 and 1B3); ligandin assembles and transport UCB to the SER; conjugation of UCB by UGT1A1 results in bilirubin glucuronides (MGB, DGB); glucuronides are water soluble and are transported to the exterior of the hepatocyte, at the apical canalicular surface, by the MRP2 (Multidrug Resistance-Associated Protein 2) and ABCC2 (ATP-Binding Cassette transporter - sub-family G member 2), and possibly by the ligandin (initially named Y protein) a glutathione-S-transferases (17), which despite being in much smaller quantity, can pass through the protein ABCC3 a ATP-Binding Cassette transporter, (Sub-Family C, CFTR/MRP, Member 3) and be again captured by OATP transporters.

After hepatic uptake and metabolism, BL may remain in the liver cells (storage) connected to cytoplasmic proteins. It can also move to the smooth endoplasmic reticulum of the hepatocyte and undergo conjugation with one or two residues of glucuronic acid (UDPGlcUA) by the catalytic action of UGT1A1 forming monoglucorunídeo (MGB) or diglucorunídeo (DGB), named as conjugated bilirubin (CB). In the gut this CB undergoes oxidation by the action of intestinal enzymes and bacterial flora and urobilinogen is formed as other pigments. The Urobilinogen may again be captured to the liver (enterohepatic circulation) and may be conjugated.

METHODS AND MATERIAL

Some studies point out that UCB may prevent cardiovascular disease (CVD) and other chronic diseases. Clinical evidence indicates that hyperbilirubinaemic individuals with GS, with mild hyperbilirubinemia, are at reduced risk of developing cardiovascular and chronic kidney disease. There are currently several studies that have established an association between low BL and the presence and severity of various cardiovascular diseases and the respective causes or co-morbidities such as, type 2 diabetes, metabolic syndrome, hypertension, chronic kidney disease and albuminuria. It was observed the same association, as described above, with other disease conditions which physiopathology is related to oxidative stress, such as rheumatoid arthritis, multiple sclerosis, cancer and overall mortality.

DRUG INTERACTION WITH BILIRUBIN METABOLISM

Many exogenous substances, xenobiotics and drugs are substrates of UGT1A1 enzyme. Genetic variations that alter the expression of UGT1A1 may be a danger as they would increase toxicity for patients. The variant most studied in this interaction corresponds to the polymorphism UGT1A1 * 28, at the UGT1A1 promoter responsible for the GS, characterized by hiperbilirubinemia. Examples of UGT1A1 substrates are irinotecan (SN-38), acetaminophen (paracetamol) and simvastatin (8).

RESULTS

An extensive variety of fruits and vegetables offer a range of nutrients and different bioactive compounds including phytochemicals, vitamins, minerals, and fibers. Many dietary compounds, present in fruits, vegetables and spices have been isolated and evaluated for their therapeutic potential. Evidence suggests that the health benefits of fruits and vegetables are attributed to the interactions of the phytochemicals present in whole foods by modulating several metabolic pathways. The impression that these compounds have health promoting effects emerged because their consumption was related to a reduced incidence of cancer, cardiovascular, neurological, respiratory, and age related diseases (21).

Table 2. Sources or dietary compounds that can modulate serum bilirubin levels by interfering with UGT1A1 activity.

Dietary compounds or dietary sources intake	Type of study	Effect on bilirubin levels Summary
Record of food intake from four botanical groups: Cruciferae, Rutaceae, Liliaceae and Leguminosae.	Observational	Decreased SBL. Only homozygotes 7/7 showed decreased bilirubin concentrations after consuming cruciferous vegetables but not with the intake of other investigated botanical groups.
Cruciferous, citrus and soy (doses adjustusd for body weight)	Clinical	Decreased SBL. The intake of cruciferous vegetables, soy foods and citrus fruit seems to be associated to a decrease on SBL but only in women that are homozygous 7/7.
Record of food intake Citrus fruit, cruciferous and soy	Observational	Decreased SBL. Women homozygous 7/7 that consume citrus fruit may exhibit a higher activity of this gene than those who do not include it on their diet.
Cruciferous, citrus and soy (Different quantities of Cruciferous supplementation)	Clinical	Decreased SBL within all of three group of vegetable and it was observed in the three genotypes. Results suggest a dose-response.
Soy	In vivo	Decreased
Resveratrol (Resveratrol doses)	Clinical	Decreased SBL. UGT1A1 activities were minimally affected by the intervention. is more pronounced in individuals with low baseline enzyme activity.
Dandelion	In vivo	Decreased
Rooibos	In vivo	Decreased
Honeybush tea	In vivo	Decreased
Rosemery	In vivo	Decreased
Ellagic acid (present in berryes, pomegranate, grapes, walnuts, and blackcurrants)	In vivo	Decreased
Ferulic acid	In vivo	Decreased
Curcumin	In vivo	Decreased
Astaxantin	In vivo	Decreased
Green tea	In vitro	Increased
Green tea	In vivo	Increased
Quercetin	In vitro	Increased
Rutin	In vitro	Increased
Naringenin	In vitro	Increased
Allspice	In vitro	Increased
Peppermint oil	In vitro	Increased
Cacao	In vitro	Increased

CONCLUSION

In a hyperbilirubinemic condition, were it important to lower serum bilirubin levels, the best approach would include the increasing UGT1A1 expression and this can be achieved with foods from the botanical families Cruciferae (e.g., broccoli), Rutaceae (citrus), Liliaceae (e.g., onions), and Leguminosae (legumes). Regulation of UGTs by phytochemicals has been investigated with a focus on cancer prevention numerous inhibitors from plant origin (epicatechin gallate, epigallocatechin gallate, octyl gallate, propyl gallate, quercetin, tannic acid, benzoin gum, capsaicin, dihydrocapsaicin, eugenol, gallocatechin gallate, geraniol, menthol, menthyl acetate, naringenin, allspice berry oil, N-vanillylnonanamide, clovebud oil, peppermint oil, silibinin, and silymarin). The strategy to rise SBL, inhibiting UGT1A1 activity appears unreasonable because UGT1A1 also glucuronides, estrogens and several dietary carcinogens.

Several studies show that low serum bilirubin concentrations are associated with an increased risk of chronic diseases, whereas slightly elevated serum bilirubin levels seems to provide protection. The enzymes HO-1 and BLV will also have an important role in the development of therapeutic strategies based on dietary compounds however for these two enzymes there was considerable less information about their inducers and inhibitors.

REFERENCES

1. Fevery J. Bilirubin in clinical practice: a review. Liver Int Off J Int Assoc Study Liver [Internet]. 2008;28(5):592–605.
2. Sticova E, Jirsa M. New insights in bilirubin metabolism and their clinical implications. World Journal of Gastroenterology. 2013. p. 6398–407.
3. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer a, Oostra B a, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med. 1995;333(18):1171–5.
4. Rodrigues C, Vieira E, Santos R, de Carvalho J, Santos-Silva A, Costa E, et al. Impact of UGT1A1 gene variants on total bilirubin levels in Gilbert syndrome patients and in healthy subjects. Blood Cells, Mol Dis. 2012;48(3):166–72.
5. Canu G, Minucci A, Zuppi C, Capoluongo E. Gilbert and Crigler Najjar syndromes: An update of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene mutation database. Blood Cells, Mol Dis [Internet]. Elsevier Inc.; 2013;50(4):273–80.
6. Rodrigues C, Costa E, Vieira E, De Carvalho J, Santos R, Rocha-Pereira P, et al. Bilirubin dependence on UGT1A1 polymorphisms, hemoglobin, fasting time and body mass index. Am J Med Sci [Internet]. 2012;343(2):114–8.
7. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for ??- tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem. 1994;269(24):16712–9.
8. Strassburg CP, Lankisch TO, Manns MP, Ehmer U. Family 1 uridine-5???-diphosphate glucuronosyltransferases (UGT1A): From Gilbert's syndrome to genetic organization and variability. Arch Toxicol. 2008;82:415–33.
9. Katoh M, Yoshioka Y, Nakagawa N, Yokoi T. Effects of Japanese herbal medicine, Kampo, on human UGT1A1 activity. Drug Metab Pharmacokinet. 2009;24(3):226–34.
10. Saracino MR, Bigler J, Schwarz Y, Chang J-L, Li S, Li L, et al. Citrus fruit intake is associated with lower serum bilirubin concentration among women with the UGT1A1*28 polymorphism. J Nutr [Internet]. 2009;139(3):555–60.
11. Peterson S, Bigler J, Horner NK, Potter JD, Lampe JW. Cruciferae interact with the UGT1A1*28 polymorphism to determine serum bilirubin levels in humans. J Nutr. 2005;135(December 2004):1051–5.
12. LC AMR. Soy feeding induces phase II enzymes in rat tissues. Nutr Cancer. 1997;28(3):270–5.
13. Condezo-Hoyos L, Abderrahim F, Conde MV, Sus??n C, D??az-Gil JJ, Gonz??lez MC, et al. Antioxidant activity of liver growth factor, a bilirubin covalently bound to albumin. Free Radic Biol Med [Internet]. Elsevier Inc.; 2009;46(5):656–62.
14. Brodersen R. Bilirubin. Solubility and interaction with albumin and phospholipid. J Biol Chem. 1979;254(7):2364–9.
15. Roca L, Calligaris S, Wennberg RP, Ahlfors CE, Malik SG, Ostrow JD, et al. Factors affecting the binding of bilirubin to serum albumins: Validation and application of the peroxidase method. Pediatr Res. 2006;60(6):724–8.
16. Hong AL, Huo D, Kim HJ, Niu Q, Fackenthal DL, Cummings S a., et al. UDP-glucuronosyltransferase 1A1 gene polymorphisms and total bilirubin levels in an ethnically diverse cohort of women. Drug Metab Dispos. 2007;35(8):1254–61.
17. Jansen T, Daiber A. Direct antioxidant properties of bilirubin and biliverdin. Is there a role for biliverdin reductase? Front Pharmacol. 2012;3 MAR.
18. Zucker SD, Goessling W. Mechanism of hepatocellular uptake of albumin-bound bilirubin. Biochim Biophys Acta - Biomembr. 2000;1463:197–208.
19. Wolkoff AW, Goresky CA, Sellin J, Gatmaitan Z, Arias IM. Role of ligandin in transfer of bilirubin from plasma into liver. Am J Physiol [Internet]. 1979;236(6):E638–48.
20. Roberts MS, Magnusson BM, Burczynski FJ, Weiss M. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. Clin Pharmacokinet. 2002;41(10):751–90.
21. Belo L, Nascimento H, Kohlova M, Bronze-da-Rocha E, Fernandes J, Costa E, et al. Body fat percentage is a major determinant of total bilirubin independently of UGT1A1*28 polymorphism in young obese. PLoS One. 2014;9(6).