Expert Opinion

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Bilirubin: The yellow hormone?

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Summary

Bilirubin is a tetrapyrrolic compound originating from heme catabolism. Although originally considered only a potentially dangerous waste product, it has become increasingly evident that this molecule represents an important modulator of various biological functions in the human body. Bilirubin appears to have versatile functions, from cell signaling (behaving almost like a "real" hormonal substance), modulation of metabolism, to immune regulation, affecting biological activities with apparent clinical and even therapeutic consequences. These activities may be the reason for the lower incidence of diseases of civilisation (cardiovascular diseases, arterial hypertension, diabetes, obesity, metabolic syndrome, certain cancers, autoimmune, and neurodegenerative diseases) observed in individuals with a chronic mild unconjugated hyperbilirubinemia, a typical sign of Gilbert's syndrome. While higher serum concentrations of unconjugated bilirubin may serve as an important protective factor against these diseases, low levels of bilirubin are associated with the opposite effect.

Keywords: Bilirubin; benign hyperbilirubinemia; Gilbert syndrome; hormone.

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Introduction

Bilirubin was long considered at best only a non-functional waste product of heme catabolism, but more often an ominous sign of liver diseases, or even a potentially neurotoxic substance. However, recent studies have shown that mildly elevated serum bilirubin concentrations, such as those typically seen in individuals with Gilbert's syndrome (GS, benign hyperbilirubinemia), but also levels in the upper quartiles of the currently accepted physiological serum bilirubin range, may protect against diseases associated with increased oxidative stress, an exaggerated immune response and metabolic dysfunction. Since the seminal work on bilirubin metabolism performed almost a hundred years ago by Hans Fisher (the only Nobel Prize laureate in this field), our view of bilirubin has dramatically changed.

Clinical aspects of bilirubin metabolism

The physiological range of serum bilirubin is conventionally defined as 5–17 μmol/L (0.3–1 mg/dl). Bilirubin has a typical bimodal distribution, due to the high prevalence of GS; thus, in cohort studies, bilirubin concentrations should always be expressed as median (IQR) instead of mean (SD). Bilirubin concentrations below 10 μmol/L (0.6 mg/dl) are associated with a higher risk of various diseases, and this is even more evident for a concentration below 7 μmol/L (0.4 mg/dl) which is still within the currently accepted physiological range.[1,2] Thus, the decision limits reflecting the health risks of these lower serum bilirubin concentrations should be established, as done for HDL cholesterol in the past.[3]

It is also well known that the serum bilirubin concentration is lower in females than males, questioning the rationale of the current physiological value of serum bilirubin concentrations not being sex-specific. Hence, independent physiological ranges should be re-established for each sex (including a lower diagnostic cut-off value of serum bilirubin concentration for GS in females).[3,4]

Hepatic bilirubin biotransformation: The key regulatory step in metabolism

Bilirubin glucuronosylation is the rate-limiting step in the disposal of unconjugated bilirubin from the human body. In humans, this process is mediated by a specific hepatic enzyme named bilirubin-UDP glucuronosyl transferase (UGT1A1, OMIM *191740), and specific mutations of the *UGT1A1* gene account for the manifestation of mild chronic unconjugated hyperbilirubinemia (GS) (Fig. 1).[5] The *UGT1A1* gene is the major gene responsible for the systemic bilirubin concentration in the general population.[6]

UGT1A1 is highly polymorphic, with about 150 allelic genotypes having been identified so far.[7] In the majority of Caucasians, GS is associated with the UDP-glucuronosyltransferase 1A1*28 polymorphism (rs8175347), an insertion of an additional TA-repeat into the promoter region of the *UGT1A1* gene, resulting in an $A(TA)_7TAA$ promoter sequence, which reduces bilirubin glucuronosylation by 70%[5] (Fig. 1). *UGT1A1**28 has an allelic frequency of 27–40% in the Caucasian population.[8] Interestingly, this allele is very rare in some populations, ranging from 0 to 5% in Melanesia and the Pacific Islands.[9] *UGT1A1**28 homozygosity is found in 16% of European, 12% of

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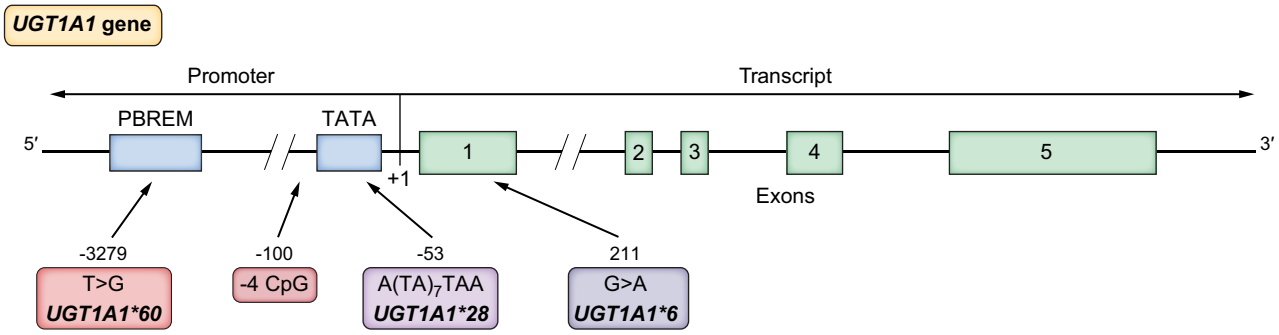

Fig. 1. ***UGT1A1*** **gene and its variations responsible for manifestation of the manifestation of mild chronic unconjugated hyperbilirubinemia (Gilbert's syndrome).**

Indian, 8% of Egyptian, and 23% of African-American individuals.[10] The penetrance of the *UGT1A1**28 homozygous mutation is only 50%,[5] most likely due to the effects of certain modifying genes[11] as well as the presence of specific haplotypes with other *UGT1A1* genotypes.[12] In addition, differential methylation of the -4 CpG site located within an upstream stimulating factor (USF) response element that regulates *UGT1A1* expression may explain a proportion of the inter-individual variability in hepatic glucuronosylation by UGT1A1 (Fig. 1).[7] All these facts highlight that GS is a phenotypic diagnosis and that *UGT1A1**28 homozygosity is only a predisposing factor.

Although Asians have been reported to have higher serum bilirubin concentrations compared to Caucasians, the $(TA)_7$ allele in that population is much less common.[13] Instead, a heterozygous mutation in the coding exon 1 of the *UGT1A1* gene (such as a presence of the *UGT1A1**6 allele) is an additional important determinant of GS in Asian populations, together with other variants in the promoter *UGT1A1* region (Fig. 1),[13] pointing to even more complex genetic regulation of bilirubin homeostasis evolving during phylogenesis.

The prevalence of phenotypic GS varies between 2–12%, being much lower in females, with a male-to-female prevalence between 2–7:1 despite the lack of significant sex differences in *UGT1A1**28 genotype frequencies,[14] pointing to the need for normal reference values of serum bilirubin to be adjusted by sex. The prevalence markedly differs among different ethnic groups, with the lowest prevalence in African Americans and the highest in Asian populations. However, caution is needed since more accurate data are lacking, owing to the absence of homogeneity in the reported population studies.[14]

Mild hyperbilirubinemia is associated with health benefits

The beneficial associations of mildly elevated systemic bilirubin concentrations with diseases of civilization (in particular those affecting the cardiovascular system) have been reported in numerous studies.[2,3,15–17] Besides, mild unconjugated hyperbilirubinemia is also associated with lower adiposity, a decreased risk of metabolic syndrome, non-alcoholic fatty liver disease (NAFLD),[18] and diabetes,[2,15] as well as overall cancer risk.[2] Bilirubin downregulates almost all functions of the immune system,[19] a phenomenon certainly implicated in the low frequency of various inflammatory, autoimmune, and degenerative diseases in individuals with GS.[2] All these observations may account for the reduced mortality rates recently reported[2] in those with GS compared to the normobilirubinemic population. Although these associations were originally ascribed to the powerful antioxidant activities of bilirubin,[20] several additional and more biologically potent activities that could account for these beneficial effects have recently been described. These activities, which can be collectively covered by the term "bilirubinomics",[17] include the modulation of cell signaling; protein phosphorylation[21]; or the activation of cytoplasmic and nuclear receptors, as well as binding to other molecules within the vascular bed and also the cell interior (Table 1), which upon complexing with bilirubin exert further biological activities (Fig. 2) (for review see[22]). Bilirubin thus acts as a 'real' endocrine molecule, with effects similar to those recently proposed for bile acids.[23] These receptors involve those implemented in energy homeostasis (such as peroxisome proliferator-activated receptors [PPARs], aryl hydrocarbon receptor [AhR], or constitutive androstane receptor [CAR]), biotransformation processes (such as CAR, pregnane X receptor [PXR]), sensitive perception (*via* MRGPRX4 (Mas-related G protein-coupled receptor X4]).[22] Also, high-affinity binding molecules, belonging mostly to a lipocalin superfamily of proteins (such as fatty acid-binding protein [FABP1] or apolipoprotein D [apoD]) (Table 1), activate, upon complexing with bilirubin, various additional cell-signaling pathways (Fig. 2).[22]

Table 1. Bilirubin as a ligand of biological targets in a human body.

Compartment	Binding to	Function*
Cell nucleus	AhR[39]	Energy homeostasis Biotransformation Immune system cell differentiation Anti-microbial effects infectious Anti-inflammatory effects Anti-atherogenic effects Inhibition of protein phosphorylation Anti-cancer effects Role in circadian rhytmicity
	CAR[40]	Energy homeostasis Biotransformation
	PXR	Biotransformation
	PPARα[41]	Energy homeostasis
	PPARγ[42]	Energy homeostasis
	apoD[43]	See below
Cytoplasm	FABP1[44]	Metabolic and immune system signaling PPARs transactivation
	apoD[43]	See below
	NADPH oxidase	Suppression of superoxide-mediated cell signaling with multiple biological effects
Cell membrane	MRGPRX4[45]	Sensitive perception Anti-inflammatory effects Energy homeostasis Anti-cancer effects Antihypertensive effects Cardioprotective activities
	apoD[43]	Anti-cancer effects Angiogenesis Immune system modulation Cholesterol metabolism Sex hormone signaling
	AFP (*via* its cell receptor)	Cell growth, differentiation, regeneration, apoptosis and transformation during onto- as well as oncogenesis
Intravascular	apoD[43]	Lipid metabolism Oxidative stress defense
	AFP	See above
	Albumin	Oxidative stress defense
	PGDS[46]	Energy homeostasis Carcinogenesis Immune system modulation
Cerebrospinal fluid	PGDS[46]	Immune system modulation

AFP, α1-fetoprotein; AhR, aryl hydrocarbon receptor; apoD, apolipoprotein D; CAR, constitutive androstane receptor; FABP, fatty acid binding protein; MRGPRX4, Mas-related G protein-coupled receptor X4; PGDS, (lipocalin-type) prostaglandin D synthase; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor. Adapted according to.[22]
*Known as well as putative functions.

Therefore, it is not surprising that mild elevation of serum bilirubin, binding to and activating a wide array of cell targets involved in cell signaling and metabolic homeostasis, is associated with lower adiposity, a lowered risk of metabolic syndrome, NAFLD,[18] diabetes,[15] and an overall better metabolic status.[24]

A hormone (from the Greek participle ὁρμῶν, "setting in motion") is any member of a class of signaling molecules in multicellular organisms that are transported to distant organs to regulate physiology and/or behavior. Hence, as we proposed previously[16] and now in detail here, bilirubin acts as a 'real' hormonal substance fulfilling most of the required criteria – being a 'chemical substance secreted into the bloodstream and acting on distant tissues, usually in a regulatory fashion upon binding to specific receptors'.[25] Our proposal is in line with the change of the classical endocrinology paradigm which has occurred during recent years with discoveries of novel hormonal substances originated from musculoskeletal, adipose, enteric, cardiac, or hepatic tissues.[26–28]

Can mild hyperbilirubinemia improve longevity? Lessons from the evolution

As mentioned above, a positive association between serum bilirubin concentrations and longevity has been reported in large human epidemiological studies (for review see[2]). In this respect, it is interesting to look at the possible evolutionary aspects of this association. The *UGT1A1* promoter in chimpanzees contains only 4 TA repeats, suggesting a much higher transcription of *UGT1A1*. This results in substantially lower systemic bilirubin levels in the great apes, where the median serum bilirubin level is lower than 1.7 μmol/L.[29] An additional interesting observation

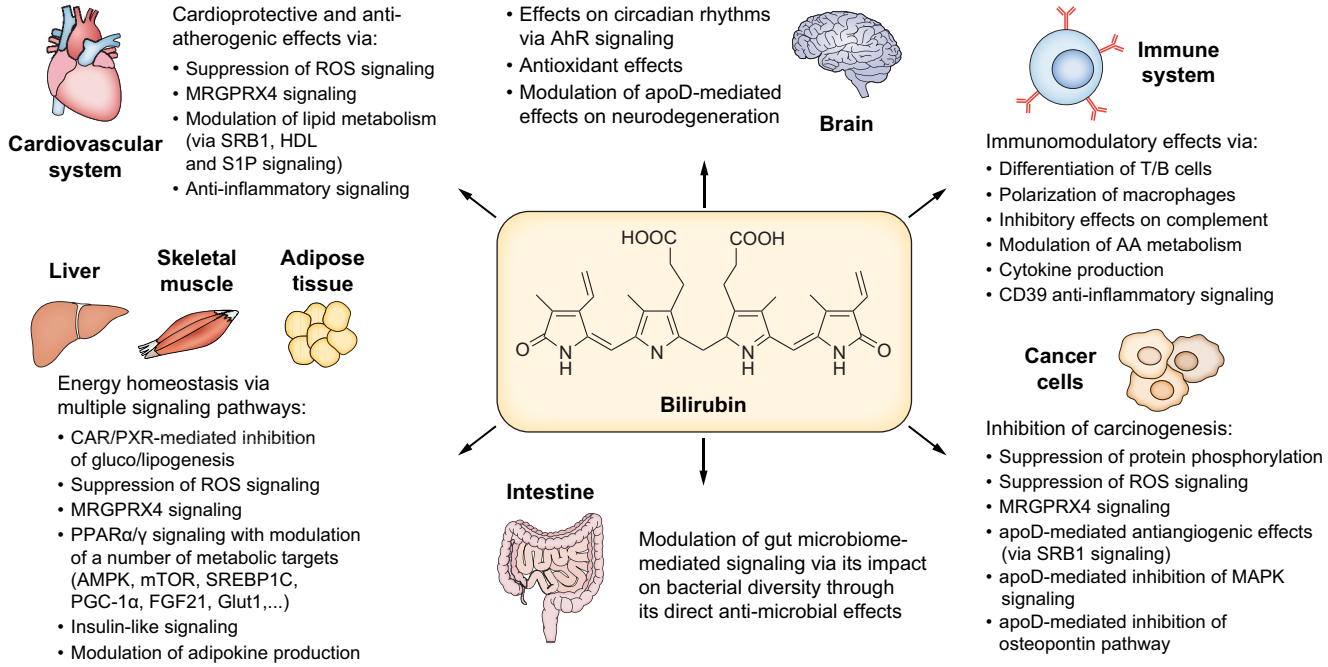

Fig. 2. Involvement of bilirubin in cell signaling pathways. This scheme represents possible involvement of bilirubin in cell signaling based on either clinically proved, experimentally proposed, or theoretically justified data reported in the recent literature. Only the key signaling pathways are illustrated. Adapted according to.[22] AA, arachidonic acid; AhR, aryl hydrocarbon receptor; AMPK, AMP-activated protein kinase; apoD, apolipoprotein D; CAR, constitutive androstane receptor; FGF21, fibroblast growth factor 21, Glut 1, glucose transporter-1; MAPK, mitogen-activated protein kinases; MRGPRX4, Mas-related G protein-coupled receptor X4; mTOR, mammalian target of rapamycin; PGC-1α, proliferator-activated receptor g coactivator 1α; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SRB1, scavenger receptor B1; SREBP-1c, sterol regulatory element-binding protein 1.

is that chimpanzees have a higher level of oxidative stress and a greater risk of cardiovascular diseases compared with humans of equivalent age,[30] and live half as long as humans, which might be due to the evolutionary acquisition of a TATA box genetic mutation in humans.[31]

As mentioned above, the lowest *UGT1A1*28* allele frequency is found in Melanesia and the Pacific islands,[9] and this is associated with significantly lower serum bilirubin levels as reported in Melanesian children[32] and adult Tongans.[33] It is tempting to speculate that the shorter lifespan in Oceania[34] might at least in part be affected by the low serum bilirubin levels due to the negative association reported between serum bilirubin concentrations and all-cause mortality (for review see,[2] and above).

Therapeutic implications of bilirubin and bilirubin-related pigments

Bilirubin behaves as a typical *yin-yang* substance, with deleterious effects at high concentrations, yet providing substantial benefits for human health when only mildly elevated, such as in those with GS (the double-faced Janus effect). This is the reason for the attempts to iatrogenically mimic GS to enhance protection against diseases of civilisation.[35] Even a tiny increase in serum bilirubin concentration seems biologically relevant since each micromolar increase (even still within the current physiological range) is associated with a significant decrease in the risk of various oxidative stress-mediated diseases.[3,16]

There are several ways to increase systemic levels of bilirubin. A decrease in the efficacy of the liver cell to conjugate bilirubin with glucuronic acid by partial inhibition of UGT1A1 was reported for numerous other xenobiotics routinely used in clinical medicine (for review see[35]), but also for naturally occurring compounds such as plant flavonolignanes.[36]

A more elegant approach to enhance the bile pigment pool seems to be dietary supplementation with bilirubin-like structures that commonly occur in Nature. One example is the blue-green algae, which contain substantial amounts of phycobilins (tetrapyrolic compounds resembling the structures of biliverdin or bilirubin[37]).

Interestingly, in the last 5 years novel therapeutic approaches for delivering bilirubin to pathologically altered tissues and organs have been investigated. Bilirubin has been incorporated into various forms of nanoparticles, and the biological efficacy of these particles has been experimentally verified in recent studies focused on the treatment of inflammatory diseases, conditions associated with increased oxidative stress or cancer.[35,38] As an example, bilirubin encapsulated into nanoparticles

was reported in preclinical studies to inhibit inflammatory processes in the pancreas as well as intestine (on a mouse model of pancreatitis and inflammatory bowel disease, respectively) *via* modulation of a wide array of effectors of both innate as well as adaptive immunity. Bilirubin was also shown to protect gut barrier integrity as well as to maintain microbiome richness and diversity, further expanding the pathophysiological implications and therapeutic potential of bilirubin.[38]

Perspectives and conclusion

From being considered a waste substance associated with liver disease, our understanding of bilirubin has come a very long way. Bilirubin is now regarded as a molecule with many intricate biological functions, while our view on mildly hyperbilirubinemic individuals with GS has changed dramatically. While in the past these individuals were regarded as predisposed to chronic fatigue and various gastrointestinal problems, often being advised against physical exercise while being kept on strict diets, it is now clear that there is no reason to label individuals with GS as “patients”. Instead, GS is likely to represent a selective advantage due to the potent beneficial biological effects of bilirubin which protect against various diseases of civilisation.

Indeed, as one of the most abundant intrinsic antioxidants in the body, bilirubin may protect us from cardiovascular disease and cancer *etc*. As always in Nature, nothing is fully good or bad, but the dark side of the coin (specifically represented by an impaired capacity to biotransform certain xenobiotics in individuals with GS, and by neurotoxicity in severe neonatal hyperbilirubinemia) is outweighed by the benefits associated with a mild elevation of bilirubin. The modulation of bilirubin concentration may be a suitable way to prevent/treat different diseases in the future; however, only time will tell whether this option is feasible and realistic.

Although the current experimental and clinical evidence strongly suggest a causative relationship between bilirubin and several clinical conditions which may be explained by its “endocrine” activities, future research is certainly needed to assess the possible contribution of reverse causality or other potential confounding factors. These may include the effects of other bioactive molecules (such as carbon monoxide formed during heme catabolism) or decreased clearance of such biomolecules due to impaired hepatic biotransformation.

The future of the “yellow research” will require the active participation of chemists, physiologists, immunologists, cell biologists, epidemiologists, and clinicians resulting in real translational research. In any case, based on the evidence presented herein and remembering the true meaning of the Greek word hormone (ὁρμῶν, "setting in motion"), we believe we can safely remove the question mark from the title, as bilirubin can certainly be viewed as “the yellow hormone”.

Abbreviations

GS, Gilbert’s syndrome; NAFLD, non-alcoholic fatty liver disease; UGT1A1, bilirubin-UDP glucuronosyl transferase.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions

Both authors contributed equally.

Supplementary data

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Author names in bold designate shared co-first authorship

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