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Molecular Insight into Receptor-mediated Therapeutic Potential of Lumirubin and 6-Formylindolo[3,2-b]carbazole in Phototherapy of Neonatal Hyperbilirubinemia

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Abstract

Objective: Neonatal hyperbilirubinemia (NNH) leading to kernicterus disturbs immune and neuronal functions through inflammation and excitotoxicity. Tryptophan metabolism along the kynurenine pathway may underpin both features. Because phototherapy of NNH converts only a small amount of bilirubin to oxidation products, the assumption is that the low levels of these oxidation products must exert highly protective effects despite the large bilirubin levels. We explored by molecular docking the likely interaction of the major photooxidation product lumirubin with enzymes and receptors involved in bilirubin toxicity. We also explored the interaction of the tryptophan photooxidation product 6-formylindolo[3,2-b] carbazole (FICZ), which may also be formed during NNH phototherapy.

Methods: We performed molecular docking of lumirubin and FICZ to the aryl hydrocarbon (AhR), N-methyl-D-aspartate (NMDA), kainate and γ-aminobutyric acid (GABA) receptors. Docking to the AhR was compared with that of bilirubin, biliverdin, indirubin and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Docking of lumirubin and FICZ to the neuronal receptors was also compared with that of their endogenous modulators kynurenic (KA) and quinolinic (QA) acids.

Results: Lumirubin and FICZ docked very strongly to the AhR, whereas biliverdin and bilirubin did not. Both lumirubin and FICZ also docked strongly to the NMDA and GABA receptors, as did KA and QA.

Conclusions: AhR activation by lumirubin may underpin its immune protection. FICZ may afford a similar protection. Interaction of lumirubin and FICZ with glutamate and GABA receptors may underpin antagonism of the excitotoxicity of kernicterus. Development of lumirubin- and FICZ-based pharmaceuticals may advance NNH therapy. Interaction of KA and QA with GABA receptors requires investigation at the pharmacological and behavioral levels.

Keywords: Bilirubin toxicity; Heme metabolism; Kernicterus; Molecular docking; Neuronal receptors; Newborn jaundice; Photooxidation; Tryptophan metabolism

Abbreviations: AhR: (aryl hydrocarbon receptor); CBS: (cystathionine β-synthetase); FICZ: (6-formylindolo[3,2-b]carbazole); GABA: (γ-aminobutyric acid); HO: (heme oxygenase); 3-HAA: (3-hydroxyanthranilic acid); 3-HK: (3-hydroxykynurenine); IDO: (indoleamine 2,3-dioxygenase); IL: (interleukin); KA: (kynurenic acid); KAT: (kynurenine aminotransferase);

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Kyn: (kynurenine); KP: (kynurenine pathway); NMDA: (N-methyl-D-aspartate);NNH:(neonatal hyperbilirubinemia); PARP: [poly (ADP-ribose) polymerase]; QA: (quinolinic acid); TCDD: (2,3,7,8-tetrachlorodibenzo-p-dioxin); Trp: (*L*-tryptophan); TDO: (tryptophan 2,3-dioxygenase); TNF: (tumor necrosis factor)

Introduction

Underdevelopment of bilirubin-uridine diphosphate glucuronosyl transferase (UDPGT) is the major cause of neonatal hyperbilirubinaemia (NNH) [1]. Severe NNH can lead to kernicterus, a condition associated with neurological dysfunction and brain damage. Bilirubin exerts its toxicity by modulating immune and neuronal functions. Thus, bilirubin causes the release of the proinflammatory cytokines interleukins IL-1β and IL-6 and tumor-necrosis factor TNF-α [2]. These cytokines are powerful inducers of the extrahepatic tryptophan (Trp)-metabolising enzyme indoleamine 2,3-dioxygenase (IDO1) [3]. IDO1 induction leads to activation of the extrahepatic kynurenine (Kyn) pathway (KP) resulting in increased production of Kyn metabolites (Figure1), of which 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid QA) undermine allogeneic T-cell proliferation and induce apoptosis, thus undermining T helper Th1 cells [4,5]. The Kyn transamination (KAT) product kynurenic acid (KA) is both pro- and anti-inflammatory [6] and is the endogenous antagonist of the N-methyl-D-aspartate (NMDA) receptors of the excitatory amino acid glutamate, in contrast with the agonist QA [7,8]. The balance between QA and KA determines the level of neuronal excitability: an important feature in kernicterus. Inhibition by bilirubin of aspartate aminotransferase [9], also known as KAT IV, raises the possibility that other KAT isoforms could also be inhibited, leading to decreased KA formation. This and the ability of IL-1β to activate kynurenine monooxygenase (KMO) and kynureninase [10] combine to drive the KP towards the above proinflammatory metabolites and shift the balance between QA and KA in favour of hyperglutamatergic activity and neuronal hyperexcitability. That acute bilirubin encephalopathy, a precursor of kernicterus, is associated with NMDA receptor activation has been reported [11]. Most brain QA is of peripheral origin and its circulating or cerebrospinal fluid levels are increased in a range of brain conditions associated with neuronal dysfunction and glutamatergic activation [12]. Bilirubin toxicity can however be modulated by the two products of the heme oxygenase 1 (HO1) reaction (Figure 1): biliverdin and carbon monoxide (CO), both of which exert protective effects: biliverdin antagonising oxidative damage and CO affording cell and tissue protection (see [13] and references cited therein). Bilirubin can also increase biliverdin levels by two mechanisms: (1) bilirubin oxidation by two cytochrome *P*-450 isoenzymes: *P*-450 2A5, which is induced by bilirubin, and *P*-450 2A6 [14]; (2) induction of HO1: an effect that can be reversed by bilirubin photoisomers [15]. Thus the balance between levels of bilirubin, biliverdin and CO is likely to determine extent of neuronal dysfunction in NNH.

Light therapy, the current gold standard NNH treatment, involves bilirubin photooxidation to less harmful or safe products. Bilirubin also possesses protective properties [9,16], but the fact that phototherapy detoxifies bilirubin suggests that the toxic effects outweigh the protective ones. The major product of bilirubin photooxidation is lumirubin [17], simple formation of which cannot explain the therapeutic outcome, as its levels are much lower than those of bilirubin. For example, whereas serum [bilirubin] in simple NNH is < 85 µM and could rise in severe cases to > 340 µM, serum lumirubin levels rise from 0.3 µM before, to only 2.5 µM after, phototherapy [18] and 11 hours after initiation of phototherapy, urinary lumirubin levels reach only 5.47 µM [19]. Lumirubin and possibly other photooxidation products must therefore exert powerful protective effects that can counteract the toxicity of considerably high levels of bilirubin. Lumirubin and other products exert no toxic effects on human neuroblastoma cells, nor influence cell cycle phase or expression of genes of bilirubin metabolism [20]. Very little is known of potential effects of lumirubin on immune markers, except its modest elevation of TNF-α protein expression at higher concentrations, compared to bilirubin, in unstimulated murine macrophage-like cells [21]. As will be discussed below, lumirubin acts as a ligand of receptors that can counteract the effects of bilirubin on immune and neuronal functions, making it a potential candidate underpinning the therapeutic activity of phototherapy. As the Trp metabolite 6-formylindolo[3,2-b]carbazole (FICZ), can also be formed by photooxidation of Trp [22,23], and given its protective effects in various disease models [24], it may play a protective role during NNH phototherapy. To explore the potential mechanisms of actions of lumirubin and FICZ in NNH, we examined in the present study using molecular docking *in silico* the likely binding of lumirubin, its 2 precursors biliverdin and bilirubin, and FICZ to a number of receptors involved in modulating immune and neuronal functions, namely the aryl hydrocarbon (AhR), N-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors, the results of which provide pointers towards the therapeutic potentials of both lumirubin and FICZ in NNH and other conditions that merit exploration at the preclinical and therapeutic levels.

Methods

Molecular docking was performed using the Molegro virtual Docker (MVD) software as described previously [25,26]. Re-ranking was performed to improve accuracy.

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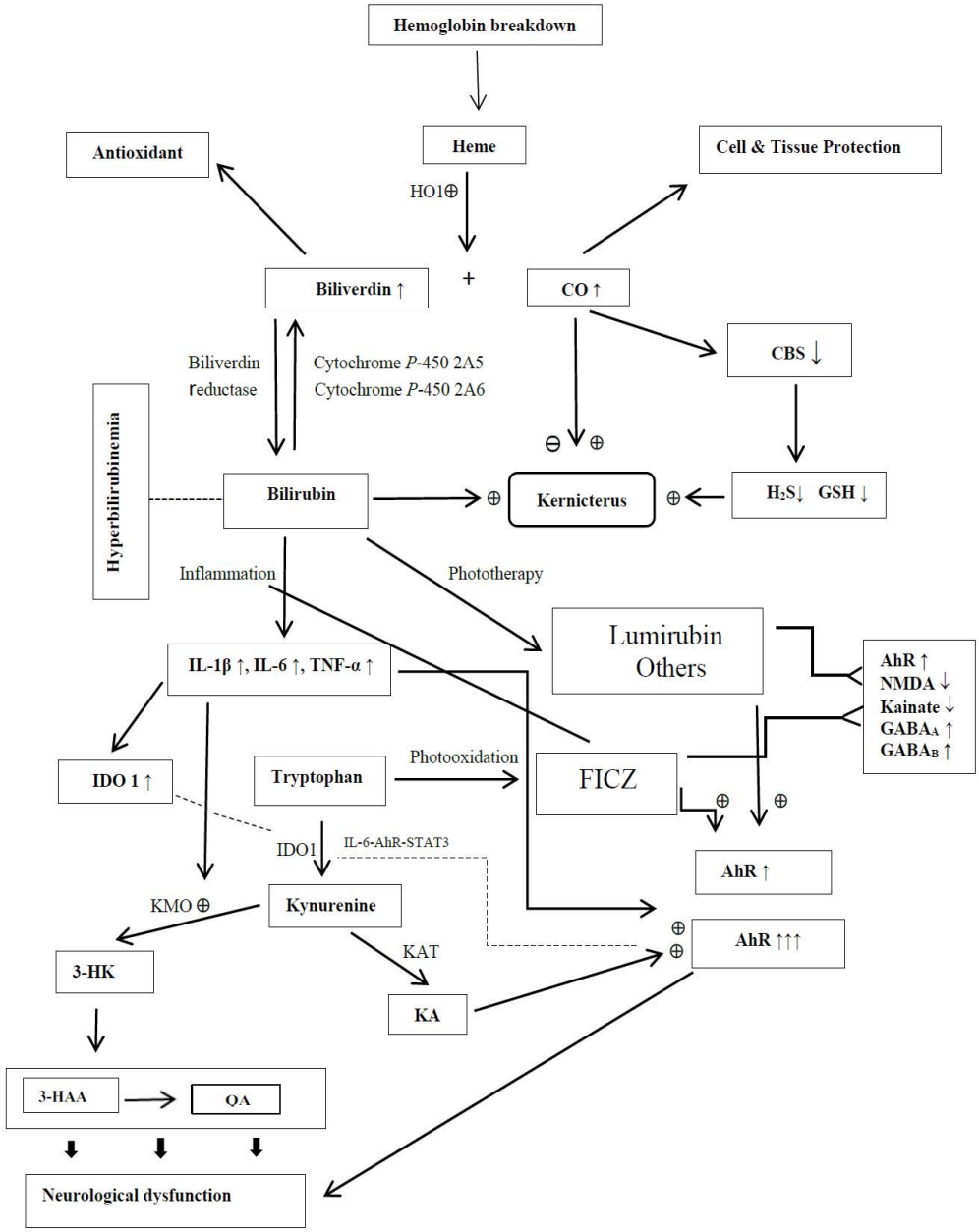

Figure 1: Diagrammatic representation of the interactions between bilirubin, tryptophan metabolism and the pathophysiology of NNH and kernicterus Abbreviations: AhR (aryl hydrocarbon receptor), CO (carbon monoxide), CBS (cystathionine β-synthase), FICZ (6-formylindolo[3,2-b]carbazole), GSH (reduced glutathione), HO1 (heme oxygenase 1), H_2S (hydrogen sulphide), 3-HAA (3-hydroxyanthranilic acid), 3-HK (3-hydroxykynurenine), IDO (indoleamine 2,3-dioxygenase), IL (interleukin), KA (kynurenic acid), KAT (kynurenine aminotransferase), KMO (kynurenine monooxygenase), QA (quinolinic acid), TNF (tumor-necrosis factor).

The heme released from hemoglobin breakdown is oxidised by heme oxygenase 1 to biliverdin plus CO. The latter two compounds exert protective effects, but CO in excess could inhibit cystathionine β-synthase activity thereby inhibiting the formation of two protective compounds: reduced glutathione and hydrogen sulphide. Thus CO could exert positive and negative effects in kernicterus. Bilirubin can be back-oxidised to biliverdin by cytochromes *P*-450. The balance between bilirubin, biliverdin and CO may thus determine the extent of toxicity to the newborn. Bilirubin toxicity elicits a proinflammatory state that can impact the kynurenine pathway of tryptophan metabolism. Cytokine induction of IDO1 results in production of kynurenine metabolites that undermine immune function and induce a hyperglutamatergic state, which can continue unabated through a vicious circle involving excessive activation of the AhR and IL-6, KA and IDO1. Phototherapy of NNH produces lumirubin, which could act by activation of the AhR, blockade of glutamate receptors and activation of GABA receptors. The Trp photooxidation product FICZ could also be formed during NNH light therapy and may act in a manner similar to lumirubin. At small rapidly metabolised levels as would be expected during light therapy, FICZ can inhibit IDO1 induction by blocking the bilirubin elevation of proinflammatory cytokines.

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When re-ranked, 10 independent docking runs resulted in 10 solutions. The structures of lumirubin, biliverdin and bilirubin (Supplementary Figure S1) were imported into the MVD workspace in SDF format. All H atoms were added and important valence checked by using the utilities in MVD. The crystal structures of a number of human receptors were obtained from the protein data bank (http://www.rcsb.org/pdb) and imported into the MVD work region at reasonable resolution levels (≤ 2.8 Å). The following receptors were tested: AhR (PDB ID: 5Y7Y [27]); NMDA (PDB ID: 3JPW [28]); $GABA_A$ (PDB ID: 4COF [29]); $GABA_B$ (PDB ID: 4MS [30]); Kainate (PDB ID: 8FWR [31]). Docking was compared between bilirubin and lumirubin with 4 enzymes of the KP, namely Trp 2,3-dioxygenase (TDO) (PDB ID: 2NW8 [32]), IDO 1 (PDB ID: 5WHR [33]), Kyn monooxygenase (KMO) (PDB ID: 5X6Q [34]) and Kyn aminotransferase II (KAT II) (PDB ID: 5EUN [35]).

The ligands were prepared by assigning the missing bonds, hydrogen(s) and charges using UCSF Chimera software. Scoring functions of the MVD and other docking details have been described [26]. Docking of the above 3 compounds to the aryl hydrocarbon receptor (AhR) was compared with that of the known potent AhR ligands FICZ, indirubin and TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin). Docking of lumirubin, its two precursors and FICZ was also performed with the NMDA, kainate, $GABA_A$ and $GABA_B$ receptors and compared with those of the well-established NMDA ligands KA and QA.

Results

Docking of biliverdin, bilirubin and lumirubin to the aryl hydrocarbon receptor (AhR)

The docking details of biliverdin, bilirubin and lumirubin to the crystal structure of the human AhR are given in Table 1 and illustrated in Figure 2. Amino acid residues at the AhR active site and those binding the ligands are listed in the legend to Figure 2. As shown in Table 1, neither biliverdin nor bilirubin docked to the AhR, whereas lumirubin docked very strongly (rerank score: -100.98 Kcal/mol). For comparative purposes, docking to the AhR of the currently recognised strong agonists indirubin (a constituent of *Indigo naturalis* and a product of bacterial metabolism of indoxyl sulphate), FICZ and TCDD is also included in Table 1. As shown, lumirubin docking was slightly stronger than those of these three powerful ligands.

Docking of biliverdin, bilirubin and lumirubin to the NMDA receptor

As summarised in Table 2, illustrated in supplementary Figure S2, and detailed in supplementary Table S1, neither biliverdin nor bilirubin docked to the NMDA receptor, whereas lumirubin docked strongly. Docking of the established NMDA ligands KA and QA was comparable, whereas that of FICZ was relatively stronger (Table 2).

Docking of biliverdin, bilirubin and lumirubin to the $GABA_A$ receptor

Neither biliverdin nor bilirubin docked to the $GABA_A$ receptor, whereas both lumirubin and FICZ did (Table 2, Figure S3 and Table S2). Both KA and QA docked at comparable energy levels, slightly lower than those of lumirubin and FICZ.

Docking of biliverdin, bilirubin and lumirubin to the $GABA_B$ receptor

As shown in Table 2, Figure S4, and Table S3, similar docking levels were observed with FICZ, KA and QA, but slightly stronger with lumirubin.

Docking of biliverdin, bilirubin and lumirubin to the kainate receptor

With the kainate receptor (Table 2, Figure S5 and Table S4), lumirubin showed the strongest docking followed by KA > QA, with FICZ being a weaker ligand. Both biliverdin and bilirubin failed to dock to the kainate receptor.

Table 1: Docking of biliverdin, bilirubin, lumirubin and other compounds to the human aryl hydrocarbon receptor.

Compound	Docking Score (Kcal/mol)	Re-rank Score (Kcal/mol)	Log P	RMSD score	Torsions	HB	Molecular weight
Bilirubin	2110.9	349.9	2.9	0	10	-9.471	584.66
Biliverdin	3141.2	507.6	2.5	0	10	-16.141	582.65
Lumirubin	-129.3	-100.9	1.4	3	3	-2.057	218.25
Indirubin	-144.1	-97.2	2.7	0	1	-3.794	262.26
FICZ	-118.1	-97.0	4.3	0	1	-1.938	282.29
TCDD	-119.8	-93.7	6.3	0	0	0	321.97

Abbreviations: TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin); FICZ (6-Formylindolo[3,2-b]carbazole); P (partition coefficient); RMSD (root mean square deviation); torsion (flexibility of ligand) HB (hydrogen bonds)

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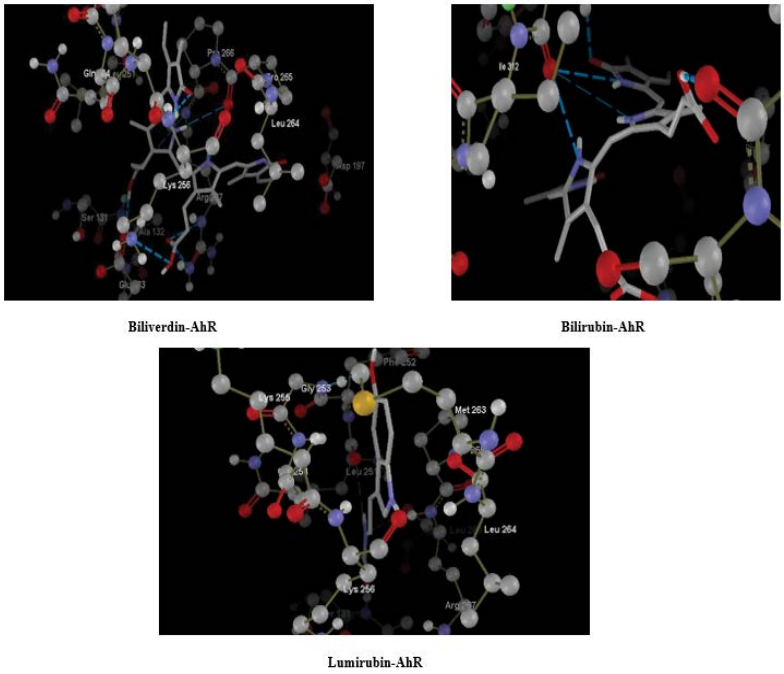

Figure 2: Docking of biliverdin, bilirubin and lumirubin to the human aryl hydrocarbon receptor (AhR). Amino acid residues at the AhR active site and those docked by ligands were as follows: biliverdin (Lys255, Arg267, Ser131, Ala132, Lys255, Gln254, Gly253, Leu251, Phe252, Pro266, Leu264) and (Lys256, Arg267) respectively; the corresponding residues with bilirubin docking were (Ser218, Val179, Ile312, Gln224, Lys313, Gly310, Phe177) and (Tyr311, Ser122) respectively; the corresponding residues with lumirubin were (Lys255, Gln254, Gly253, Leu251, Phe252, Pro266, Leu264) and (Gly253, Lys256) respectively.

Table 2: Rerank docking scores of biliverdin, bilirubin, lumirubin, FICZ, KA and QA to various receptors.

Re-rank score (Kcal/mol)				
Receptor ….	NMDA A	Kainate	$GABA_A$	$GABA_B$
Compound				
Biliverdin	446.2 (-)	648.3 (-)	398.5 (-)	390.9 (-)
Bilirubin	345.3 (-)	818.3 (-)	413.8 (-)	371.5 (-)
Lumirubin	-89.6 (*Ant*)	-96.5 (*Ant*)	-62.6 (*Ago*)	-71.6 (*Ago*)
FICZ	-95.6 (*Ant*)	-13.8 (*Ant*)	-65.6 (*Ago*)	-61.5 (*Ago*)
KA	-72.6 (Ant)	-93.2 (Ant)	-54.7 (*Ago*)	-60.5 (*Ago*)
QA	-74.9 (Ago)	-75.9 (Ago)	-53.5 (*Ant*)	-58.4 (*Ant*)
Text in italics indicates a possible position based on indirect experimental observations.				

Docking of bilirubin and lumirubin to key enzymes of the kynurenine pathway

As shown in Table 3 and depicted in Figure 3, bilirubin failed to dock to any of the 4 KP enzymes Trp 2,3-dioxygenase (TDO), indoleamine 2,3-dioxygenase (IDO1), Kyn monooxygenase (KMO) and Kyn aminotransferase II (KAT II), whereas lumirubin docked strongly to all 4 enzymes.

Table 3: Re-rank docking scores of bilirubin and lumirubin to enzymes of the kynurenine pathway.

Re-rank score (Kcal/mol)		
Compound …. **Enzyme**	**Bilirubin**	**Lumirubin**
TDO	554.8	-72.1
IDO1	440.4	-98.5
KMO	364.3	-82.4
KAT II	369.6	-80.6

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Discussion

Neonatal hyperbilirubinemia (NNH) is due to underdevelopment of bilirubin UDPGT [1], with phototherapy being the current standard treatment. The use of adjunctive phenobarbitone (an inducer of bilirubin UDPGT) makes little difference to the efficacy or speed of action of light therapy [36]. Whereas it is generally accepted that light therapy neutralises the toxicity of large concentrations of bilirubin and that the main photooxidation product lumirubin is not toxic, but can afford protection to the newborn infant, the precise mechanism(s) of its action is not fully understood. In the present study, we provide evidence from molecular docking *in silico* that lumirubin is one of the most powerful AhR ligands known, exhibiting a docking (binding) score greater than those of the potent ligands FICZ, indirubin and TCDD (Table 1). We also demonstrate the failure of bilirubin and biliverdin to dock to the crystal structure of the human AhR, thus providing the first test of direct interaction (or lack of it) of these 2 heme-degradation products with this receptor since 1998 and emphasising the need to assess binding of ligands to target proteins by the use of more direct pharmacological methods (see [16] and also the discussion on indoles [37]), rather than the widely used proxy system of *P*-450-related drug metabolism (see, e.g,[38]). Other alternative methods include the use of $AhR^{-/-}$ KO models or molecular docking. Only the latter method tests ligand-protein interaction in the purest form free from the biological confounders that exist in cell cultures, tissue preparations or *in vivo*. As far as we could ascertain, ours is the first study to demonstrate by molecular docking the binding to the NMDA receptor of its main physiological modulators the antagonist kynurenic acid and agonist quinolinic acid and that both lumirubin and FICZ exhibit strong ligand binding to these receptors. We therefore suggest that a biological basis could be proposed for a role of lumirubin and possibly also FICZ in NNH phototherapy. In the following discussion focusing on immune and neuronal dysfunction in NNH, we examine the potential mechanisms of the proposed efficacy of these two light-induced compounds based on their known properties and their interactions with receptors.

Modulation of immune function

The aryl hydrocarbon receptor (AhR) is a transcription factor, activation of which can be beneficial, whereas its over activation is harmful [39]. It is described as "a sensor that modifies the immune response to the prevailing situation: reducing inflammation in an endogenous response situation, and, in the face of an exogenous threat, limiting damage by enhancing proinflammatory Th17 cell differentiation, antiinflammatory IL-22 generation and *P*-450-dependent metabolism of the foreign agent" [39]. For example, in experimental models of inflammation, the potent AhR agonist FICZ can either ameliorate or enhance severity. It can afford protection by decreasing levels of proinflammatory IL-17, IL-1β, IL-6, IFN-γ and TNF-α and increasing those of IL-10 and IL-22, but it can also increase severity by promoting Th-17 cell differentiation (see [40] and references cited therein). These opposite effects of FICZ are determined by the route of administration and hence pharmacologically acting levels, with oral or parenteral administration being associated with decreased severity, presumably due to faster clearance and consequently low levels, and the subcutaneous route favouring slow and sustained absorption allowing continued activation of the AhR to induce a greater severity (see [24,40] and references cited therein). However, as phototherapy does not cause harm (see below), it is likely that during NNH phototherapy FICZ will not accumulate in large enough concentrations. The same applies to lumirubin especially in view of its rapid clearance [41] and albumin binding, however limited this is in infants. As is the case with FICZ, lumirubin can however exert toxic effects, but this has been demonstrated with a concentrations (25 μM) described by Jašprová et al. [42] as biologically-relevant, although the effects of lumirubin on proinflammatory cytokine gene expression were cell-dependent. Earlier, the same group [15] reported the inability of lumirubin at the above high concentration to influence the viability of SH-SY5Y cells, unlike bilirubin. As stated above, the rise in serum and urinary lumirubin after phototherapy is in the low μM range [18,19].

Although bilirubin does not interact with the AhR, it can activate it indirectly via proinflammatory Kyn metabolites after cytokine induction of IDO1. KA is the Kyn metabolite with the greatest affinity for the AhR [43]. One such cytokine whose levels are elevated by bilirubin, TNF-α, enhances gene expression of IL-6 [44]. IL-6 participates in the AhR control of IDO1 expression via the autocrine loop of AhR-IL-6-Stat3 signaling [45,46] and, although KA induces IL-6, the elevation of this cytokine by inflammation can induce IDO1 to produce sufficient amounts of KA to activate the AhR [43], thereby establishing a vicious circle. Bilirubin toxicity is associated with these changes [9]. The AhR also controls expression of poly (ADP-ribose) polymerase (PARP) that is involved in many important processes, including DNA repair [47,48]. A potential activation of NAD^+-consuming PARP can result in cell death due to NAD^+ and ATP depletion [49,50]. The preceding discussion suggests the need to assess the ability of lumirubin to counteract the above effects of bilirubin. Lumirubin however may act directly on enzymes of the KP, as our docking data (Table 3, Figure 3) suggest. It interacts with the 4 key KP enzymes TDO, IDO 1, KMO and KAT, but in which direction remains to be established in studies of enzyme activities and metabolite levels. Given its protective effect, lumirubin is likely to act as antagonist (inhibitor) of IDO1, TDO, KMO and possibly also KAT. Part of the lumirubin structure resembles that in a number of IDO1 inhibitors (see, e.g., [51]). If lumirubin is established as a dual

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TDO/IDO1 inhibitor, it may qualify as a potential cancer therapy, given the involvement of these 2 enzymes in cancer biology [52]. FICZ has already been shown in experimental cancer models to exhibit therapeutic potential [53-55].

Modulation of neuronal function

As the excitotoxicity in kernicterus involves glutamatergic activation, we have explored the possibility that docking to NMDA, kainate and GABA receptors by the above compounds may provide pointers to their toxic or protective effects. As neither bilirubin nor biliverdin interacts directly with the NMDA, kainate or GABA receptors, it is most likely that bilirubin-induced neuronal dysfunction is an indirect effect probably mediated by modulation of Trp metabolism by proinflammatory cytokines creating a hyperglutamatergic state that would be reflected in an increased [QA]/[KA] ratio and excitotoxicity. By contrast, lumirubin interacts directly with the above receptors, thereby providing a biological basis of its efficacy during NNH phototherapy against neuronal dysfunction. Whereas docking of KA and QA to the NMDA, $GABA_A$ and $GABA_B$ receptors was comparable, that of lumirubin was moderately greater (by 22.0%, 18.5% and 20.5% respectively). Given the opposite effects of KA and QA on NMDA receptors and the decreased toxicity of bilirubin upon photooxidation, it is not unreasonable to suggest that lumirubin may act as an antagonist of NMDA and kainate receptors. As FICZ can also be formed by photooxidation of Trp, it is most likely to act as antagonist of NMDA and possibly also kainate receptors.

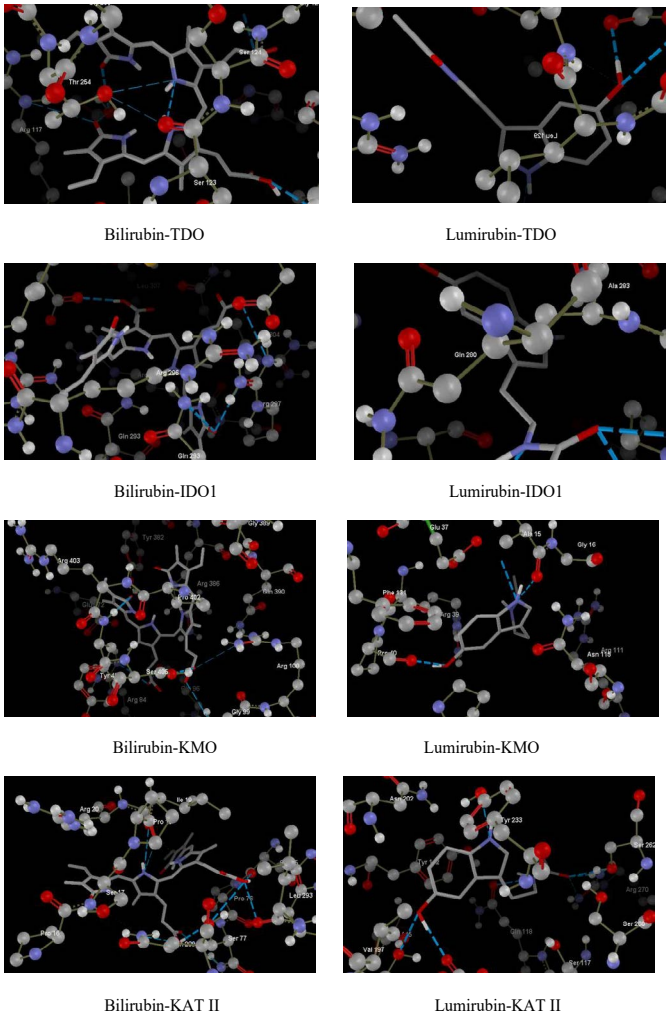

Figure 3: Docking of bilirubin and lumirubin to kynurenine pathway enzymes

Amino acid residues at the enzyme active site and those binding the ligands were as follows: TDO: bilirubin (Thr254, Arg117, Ser123,124 and Gly125, Thr254, Ser124 respectively), lumirubin: (Leu129, 30, Gln130, Arg158, Ser128 and Ser128, Asp31) IDO 1: bilirubin (Arg296,297, Gln293, Arg303, Leu307 and Arg297, Gln293), lumirubin (Gln280, Ala283, Glu119, Gly284, Arg297 and Arg297, Thr 282). KMO: bilirubin (Pro402, Ser405, Arg386, Gln390, Tyr382, Arg403 and Arg100,84, Gly99), lumirubin (Arg111, Asn115, Gly16, Ala15, Arg39, Glu37, Phe13 and Phe131), KAT II: bilirubin (Ser17,77, Pro76, Gln289, Ile19, Pro18, Arg20 and Ala78, Ser17), lumirubin (Tyr233,142, Asn202, Ser117,260, Gln118 and Ser262, Tyr233, Arg270).

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Interpretation of our docking results of KA and QA to GABA receptors is however more complex. That KA docking to $GABA_A$ may indicate an agonist behaviour is suggested by the ability of bicuculine (a $GABA_A$ antagonist) to inhibit KA-enhanced swimming and climbing of mice in the forced-swim test [56]. That KA may also act as a $GABA_B$ agonist is suggested by the similar behavior of KA produced by KMO inhibition [57] and the $GABA_B$ agonist baclofen [58]: that of inhibition of drug-seeking. That KA does not act as a GABA antagonist is further suggested by a study in hippocampal slice preparations [59] and the slowing down of the decay kinetics of $GABA_A$-evoked currents in cultured neurons [60]. Depression of GABAergic neurotransmission can be mediated by activation of kainate receptors and, as a kainate receptor antagonist, KA has been reported to reduce the kainate-dependent disinhibition by synaptic glutamate release [61]. By contrast, activation of glutamate receptors by QA should exert the opposite effects on GABAergic neurotransmission, namely antagonism of both $GABA_A$ and $GABA_B$ receptors. That QA may act as a $GABA_A$ and $GABA_B$ antagonist is suggested by the increased density of $GABA_A$ receptors following QA lesions in striatum [62] and the ability of baclofen to ameliorate the effects of QA on pyramidal neurons of the rat CA1 sector of dorsal hippocampus [63].

With lumirubin and FICZ, their potential effects on the above receptors have not been studied and should therefore be the subject of future investigations. FICZ is almost certain to be produced from Trp during NNH phototherapy. In adult plasma, Trp exists largely (90-95%) bound to albumin. The low albumin in the newborn infant results in increased free Trp availability. At birth, both free and total (free + albumin-bound) [Trp] in the newborn infant are double those in maternal circulation (see [64] and references therein). Light therapy is almost certain to convert some Trp into FICZ in infant tissues including the brain. FICZ possesses a range of properties [24]. The effects of FICZ on immune function are complex, multifactorial and dose-dependent. FICZ at low protective levels, as would be expected during phototherapy, can induce an antiinflammatory state [39] to counter bilirubin toxicity.

Conclusions and Comments

Exploring the formation of FICZ and lumirubin and their actions during phototherapy of NNH may represent a novel approach in understanding the pathophysiology and therapy of the newborn jaundice. The interactions between Trp and bilirubin metabolism and the potential mechanisms involved are illustrated in Figure 1. Whereas lumirubin may protect the newborn infant through its binding to the AhR, mainly by reducing inflammation [20], its potential effects on neuronal function remain to be explored. Studying the effects of lumirubin and FICZ on Trp metabolism along the KP in relation to immune and neuronal functions coupled with assessments of cytokine and chemokine levels and glutamatergic activity in experimental settings and where appropriate in the newborn infant should provide a platform for understanding the pathophysiology and neurological features of NNH and kernicterus and point towards pharmacological therapeutic options. Exploring IDO1 induction and increased KP metabolites in kernicterus may yield important information. The present molecular docking findings of direct interactions of KA and QA with GABA receptors are novel observations worthy of experimental scrutiny at the pharmacological and behavioral levels. The present study illustrates the value of molecular simulation in revealing potential biological and therapeutic mechanisms.

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