

ANSWERING REVIEWERS

July 12, 2013

Dear Editor,

Please find enclosed the edited manuscript 3411.

Title: New insights in bilirubin metabolism and their clinical consequences

Authors: Eva Sticova, Milan Jirsa

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3411

The manuscript has been improved according to the suggestions of reviewers:

1. Reviewer 02447096

The review by Sticova et al. reports an interesting review about bilirubin metabolism and possible clinical consequences. The authors well reviewed the importance of bilirubin and the new role as something else than an end product of haeme breakdown. In addition, they showed an extensive review of multiple syndromes that cause a hyperbilirubinemia. The review is well written and details a well conducted study.

A few suggestions to the authors:

1. Page 4 line 5: (Figure). Needs to indicate Figure 1

Corrected as suggested by the reviewer.

2. Page 4 line 29: “the Bolibian population”. Bolibian doesn’t need capital letter.

According to the original article (Ref. 51) and the opinion of our language editor capital letter in “Bolivian” is possible in this context.

3. Page 5 line 2: “anion excretion defect (TR -/-), Eizai hyperbilirubinemia rats (EHBR), mutant Corriedale, and Mrp2 (-/-) mice.....”. The authors must change , by ;

The original text has been changed as follows:

These include the Gunn rat and Ugt1(-/-) mouse mimicking the Crigler-Najjar syndrome type I^[49, 50, 51], the Bolivian population of squirrel monkeys mimicking Gilbert syndrome^[52, 53] and mutant TR or GY (Groningen yellow) rats with organic anion excretion defect (TR -/-), Eizai hyperbilirubinuria rats (EHBR), mutant Corriedale sheep, and Mrp2(-/-) mice, all modelling the Dubin-Johnson syndrome^[54-58, 112].

4. Page 10 line 29: “identified.Furthermore....”. Is necessary an space.

The space has been inserted in the revised version of the draft.

5. Page 10 line 30: “has been identified..”. The authors must eliminate a point.

The redundant point has been removed.

6. Conclusions and perspectives: Is necessary indicated the corresponded bibliography that supports the explications and must be more extended.

The list of references has been extended as suggested by the reviewer.

2. Reviewer 00006675

This is a very interesting review on the molecular mechanisms involved in the liver handling of bilirubin and in the associated genetic diseases as well as in cholestasis. The paper is clearly written and contains valuable information.

Major comments:

1. In the last paragraph of page 3 and the initial one in page 4, the authors have mentioned the carriers involved in conjugated bilirubin transport and the role of mouse ortologues of OATPs in bilirubin uptake, however a reference regarding the ability of human forms of these transporters to carry out the uptake of unconjugated and conjugated bilirubin is missing (see Briz et al., Biochem. J. (2003) 371, 897–905).

We gratefully acknowledge the valuable comment of the reviewer and the former text has been supplemented as follows:

Furthermore, results of functional studies demonstrate that OATP1B3, but not OATP1B1, may play an important role in the carrier-mediated uptake of foetal UCB by the placental trophoblast and contribute to elimination of UCB across the placental barrier^[25, 26].

2. Although the review would improve its clarity by a scheme on transport and metabolic processes, this should be accurate and complete. The text and Figure 1 is misleading and must be corrected. The expression of MRPs at the basolateral membrane is quite low under physiological circumstances and hence the relevance of the intra-parenchymal cycle of bilirubin is unclear.

We agree with the reviewer that expression of MRPs at the basolateral membrane is quite low under physiological conditions. Nonetheless, the latest results of a series of animal experiments conducted by Dr. Alfred Schinkel and coworkers clearly demonstrate existence of a sinusoidal liver-to-blood shuttling loop of bilirubin and possibly other substrates and provide new information clarifying an important role of Abcb3 and OATP1B1/1B3 in the process of hepatic clearance of bilirubin (see Ref 18, 19, 22, 125). The results of studies with Oatp 1a/1b-null mice and Oatp1a/1b;Abcc3 combination knockout mice have completely challenged the former view that it is only under pathological conditions characterized by impaired biliary excretion, that ABC efflux transporters will transport bilirubin glucuronides back from hepatocytes into the sinusoidal blood. The results of the

experiments plainly demonstrate that even under physiologic conditions, a substantial portion of bilirubin glucuronides is not excreted into bile but is transported back to the blood by Abcc3. Oatp1a/1b activity accentuated in a downstream (centrizonal) hepatocytes allows efficient reuptake of bilirubin conjugates, with a subsequent possibility being safely eliminated by excretion into bile. This suggests that OATP1B transporters and ABC efflux transporters form a sinusoidal liver-to-blood cycle mediating the process of bilirubin and other substrates shifting (hopping) from periportal to centrizonal hepatocytes as is demonstrated by our scheme.

3. Figure 1. Please check the nomenclature and spelling of conjugated bilirubin.

Corrected to bilirubin diglucuronide.

4. ABCG2 is shown in Figure 1 but its role in bilirubin transport is not mentioned/cited in the text.

In agreement with the reviewer's comment we have changed the original text as follows:

The excretion of conjugated bilirubin into bile is mediated by an ATP-dependent transporter identified as the multidrug resistance-associated protein MRP2/cMOAT and, to a lesser extent, by ATP-binding cassette (ABC) efflux transporter ABCG2.

3. Reviewer 00742205

This is a well-written review article with only a few minor concerns.

1. page 4 2nd paragraph: Recent data have ---. However, two references were published almost 20 years ago.

In agreement with the reviewer's comment we have changed the original text as follows:

Mild or moderately elevated serum bilirubin seems to be beneficial: Bilirubin is known as a strong antioxidant^[27, 28] and the protective effects of bilirubin on atherogenesis and cancerogenesis have been demonstrated in both *in vitro* and *in vivo* studies^[29-33].

2. GS is an important contributing factor to breast milk jaundice which should be mentioned.

Contribution of GS-associated mutations to breast milk jaundice has been mentioned in the revised text as suggested by the reviewer:

GS is benign and GS carriers present with no liver disease. However, the mutations in the *UGT1A1* identical to those recognised in GS subjects may contribute to the development of prolonged neonatal hyperbilirubinemia in breast-fed infants^[93, 94].

3. page 8 paragraph 2 ---excretion of total coproporphyrin -- it should be coproporphyrin

Spelling has been corrected.

4. DS shows typical biphasic BSP levels in blood which should be mentioned.

Biphasic BSP clearance in DJS subjects has been mentioned in the revised text as follows:
BSP clearance in DJS subjects is normal at 45 min with the second peak at 90 min^[106].

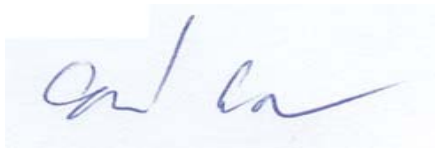
5. page 10 PBC should be spelled-out the first time.

Corrected in the revised text.

References, typesetting and style were checked and corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in blue ink, appearing to read 'E. Sticova', is shown on a light blue background.

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