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Blood 142 (2023) 2444–2445

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Biliverdin Reductase B Protects Against Primaquine Induced Hemolysis - Identification of a Novel Anti-Oxidant Pathway in Murine Erythrocytes

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Background: Biliverdin Reductase B (BlvrB) is the fourth most abundant enzyme in red blood cells (RBCs) and is widely expressed in other tissues. In normal heme catabolism, porphyrin is converted to biliverdin-alpha (by hemoxygenase (HO)) and then reduced to bilirubin-alpha (by biliverdin reductase A (BlvrA)). Bilirubin-alpha is then glucuronidated and excreted. The large body of literature investigating heme metabolism, bilirubin-based pathologies, and the oxidative biology of bilirubin/biliverdin in adults focuses almost exclusively on BlvrA and the alpha isomers of biliverdin and bilirubin. However, porphyrin is a tetrapyrrole with 4 distinct meso-carbon bridges, each of which can be broken to generate a distinct biliverdin isomer (alpha, beta, gamma, or delta). Although HO is exquisitely specific for the alpha-bond (generating only biliverdin-alpha), chemical coupled oxidation of heme yields all four biliverdin isomers. BlvrB (and not BlvrA) can reduce each of non-alpha biliverdin isomers into their corresponding bilirubin isomers (bilirubin-beta is prevalent in neonatal jaundice and kernicterus). BlvrB also reduces flavins and additional quinone metabolites. Despite BlvrB's abundance and evolutionary conservation, its only known function is a subtle effect on hematopoietic lineage fate (megakaryocyte vs. erythroid). BlvrB has no known function in mature RBCs. We hypothesized that BlvrB plays an important antioxidant role that would only become obvious when other redundant anti-oxidant pathways were limited.
Methods: A novel BlvrB knockout mouse was generated on a C57BL/6 background. A model of primaquine (PQ) induced oxidative hemolytic anemia in G6PD deficient mice was used as a clinically relevant scenario in which compromised antioxidant biology leads to treatment limiting pharmacotoxicity in RBCs. BlvrB-KO mice were crossed with two strains of mice that had murine G6PD replaced with either the A- deficient variant of human G6PD (hG6PD(A-)) or the non-deficient form of human G6PD (hG6PD(ND)). Four mouse strains (i.e., hG6PD(ND) and hG6PD(A-) mice with or without BlvrB deletion) received either a 4-day course of PQ or control PBS injections. Anemia was monitored by hematocrit (HCT). Reticulocyte count (RET) was monitored by flow cytometry as CD71+, thiazole orange+ erythrocytes. Peripheral blood was subjected to high resolution metabolomics to elucidate biochemical changes.
Results: All mouse strains were healthy at baseline, with normal measured hematological parameters (i.e., Hct 40, RET 1.5%). No change in HCT or RET was observed in hG6PD(ND) or BlvrB-KOxhG6PD(ND) mice receiving PQ or in any strain receiving PBS. PQ induced hemolytic anemia in hG6PD(A-) mice, with an average HCT of 32.7 (18% drop) with RET of 3.1%. A significant exacerbation of PQ induced hemolysis was observed in BlvrB-KOxhG6PD(A-) mice that had an average HCT of 22.5 (44% drop) with RET of 5.3%. (n= 11 mice per group, p <0.001 between groups). Blood from PQ-treated hG6PD(A-)xBlvrBKO mice showed defective glycolysis at the level of glyceraldehyde 3-phosphate dehydrogenase, increased levels of multiple long-chain acylcarnitines, altered redox metabolism, and specific increases of hypoxanthine and sphingosine-1-phosphate (S1P). Three separate experiments were performed with similar results.
Discussion: These data demonstrate that BlvrB deletion exacerbates oxidative injury and suggest that BlvrB provides a novel and hitherto undescribed antioxidant pathway in RBCs. Because approximately half a billion humans exhibit some form of G6PD deficiency, these data are also directly relevant to a large population of humans, which were modeled using hG6PD(A-) mice. These studies also define the metabolic lesion in peripheral blood. For example, GAPDH is well known to be highly sensitive to inactivation by oxidative damage, resulting in impaired glycolysis. Changes in hypoxanthine and S1P are known to be associated with decreased RBC function and survival. Finally, altered carnitine metabolism is associated with repair of

lipid peroxidation in RBCs via the Lands Cycle. Although the mechanism by which BlvrB facilitates antioxidation is not yet clear, we hypothesize it involves redox cycling of non-alpha isomers of biliverdin and bilirubin. However, the mechanisms may be through reduction of flavins and/or other endogenous substrates. Ongoing studies are testing these hypotheses.

Disclosures D'Alessandro: *Macopharma:* Consultancy; *Hemanext Inc:* Consultancy; *Omix Technologies Inc:* Current equity holder in private company. **Zimring:** *Rubius Therapeutics:* Consultancy.

https://doi.org/10.1182/blood-2023-178655