Reply to Sedlak and Snyder: The Little Bighorn of the Biliverdin Reductase Amplification Cycle

This is a response to a letter by Sedlak and Snyder (1).

Sedlak and Snyder circle their wagons for biliverdin reductase (BVR) by staking up front as proven fact the hypothesis that is being tested, and they finish with the same article of faith. Although bilirubin (BR) has antioxidant activities in vitro (2), it is less clear whether the pigment is a major physiological antioxidant for cells in vivo and if so whether regeneration of bilirubin (BV) by BVR is involved, as hypothesized by Snyder and co-workers (3). Maines and colleagues (4) indeed reported that BV depletion accentuates cellular toxicity by arsenate, but this was independent of BR or the reductase activity of BV or hence cannot have involved, nor directly supported, the BVR redox cycle.

Our studies (5) do not support the results of Snyder and associates relevant to the BVR redox cycle (3). Regarding H₂O₂ concentration, we used 0–2000 μM whereas Snyder and co-workers used 0–1000 μM, and we observed higher concentrations (>500 μM) to be required for cell death to occur (5) compared with Snyder and colleagues (100 μM) (3). However, BVR depletion also failed to affect the death of cells loaded with BV or BR prior to exposure to a 40-fold molar excess of H₂O₂ (5), whereas Snyder and associates reported BR to be able to defend against a 10,000-fold excess of H₂O₂ (3). Thus, use of overwhelming H₂O₂ concentrations in our study (5) is not likely to explain the discrepant results. We cannot rule out that a difference in exposure time of cells to H₂O₂ contributed to the varying results. However, the notion that BVR protects cells from death only at 18–24 h post-oxidant is inconsistent with the BVR cycle because cells contain BR at the time of oxidant addition as a result of constitutive expression of heme oxygenase 2 and BVR. In the case of BVR overexpression, failure to protect cells from H₂O₂-induced death was also seen in cells loaded with supraphysiological concentrations of BV or BR (5). Therefore, a limited supply of the BVR substrate or indeed BR, as suggested by Snyder and Sedlak (1), cannot explain the different results.

Regarding yields of BV formation during BR oxidation, we placed importance on physiological relevance rather than favorable conditions. Snyder and associates (3) confirmed our original observation (6) that albumin-bound BR gives the most favorable results, but albumin-bound BR is an inappropriate model for cellular BR because albumin is not a major cell protein. In contrast, we used BR added to cells, bound to glutathione S-transferase (GST), or dissolved in chloroform or dimethyl sulfoxide (DMSO) (5). We consider these relevant models of cellular BR because we used cells instead of albumin, GST is a known cellular binding protein for BR, and the two solvents reflect the different conformations of the scarcely water-soluble pigment in vivo that is determined by the extent of its intramolecular hydrogen bonding (7). We observed at best modest yields of BV formation, irrespective of the conditions and oxidants used (5). Our findings fully corroborate numerous reports (e.g. Refs. 2, 6, 8, 9), and they establish that, in general, most BR undergoing oxidation yields products other than BV. In vivo, cells contain a multitude of antioxidants. The non-enzymatic antioxidants GSH, ascorbic acid, and tocopherol are present at concentrations several orders of magnitude greater than BR, and kinetic studies indicate (10–12) and competition experiments show (2, 6) that under conditions most favorable for BR, the pigment at best just exceeds the radical scavenging activity of these antioxidants, even when comparisons are done at equimolar antioxidant concentrations. Similarly, kinetic considerations indicate that enzymatic antioxidants like glutathione peroxidases and peroxiredoxins greatly outcompete BR for reaction with H₂O₂. Thus, it is improbable that direct oxidant scavenging by BR significantly contributes to physiologically relevant cellular protection by the pigment, irrespective of the yield of BV formation or the presence of BVR.

In summary, our data (5) and chemical considerations do not support several key assumptions and prerequisites for the proposed BVR redox amplification cycle (3). Therefore, in our opinion, the latter should be treated as an unproven hypothesis rather than established fact.

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