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Viewpoint

Brightening the Path: Riboflavin Illuminates Breast Cancer Resistance Protein Monitoring

The membrane transporter breast cancer resistance protein [BCRP, also known as ATP-binding cassette superfamily G member 2 (ABCG2)] controls drug delivery, absorption, and drug-drug interaction (DDI) (Maliepaard et al., 2001), with important implications in the pharmacokinetics and drug development of a vast number of compounds used for a range of diseases including cancer. First discovered in the breast cancer cell line MCF-7/AdrVp (Doyle et al., 1998), BCRP is also expressed in physiologic conditions in numerous tissues including the apical membrane of enterocytes, hepatocytes, kidney proximal tubules, placenta (Maliepaard et al., 2001; Fetsch et al., 2006), and the lactating breast, where during pregnancy BCRP increases significantly and is responsible for the secretion of drugs, toxins, and vitamins including riboflavin (vitamin B2) into the milk (van Herwaarden et al., 2007). In addition to its critical functions in healthy states, BCRP overexpression in some cancers is one of the driving factors responsible for the failure of numerous therapies due to DDI and drug resistance. In fact, BCRP is a molecule likely responsible for multidrug resistance of tumor cells (Cascorbi, 2006). The discovery of BCRP inhibitors including curcumin, pantoprazole, and rolapitant has provided encouraging results indicating that modulation of BCRP increases the levels of sulfasalazine, a known BCRP substrate (Zaher et al., 2006). The typical approach to identify BCRP inhibitors has combined prediction models generating candidate molecules with validation in in vitro systems and subsequently in clinical trials (Feng et al., 2014; Costales et al., 2021). However, some of the limitations of this approach include high rates of false-positive predicted molecules and lack of translational value. False-positive molecules are usually found in predictive models based on cell lines that indicate a DDI, but these results are not validated further in animal models or clinical trials (Chu et al., 2018; Arya et al., 2022), a remarkable use of resources and time for potential candidate molecules that needed to be improved. In fact, more recent prediction models designed to identify endogenous biomarkers have revealed a much more accurate and successful approach able to significantly improve the pharmacokinetics of numerous new and already-in-use drugs and limit the rate of false positives.

Using metabolomics in mice deficient for the two major intestinal drug transport proteins, Bcrp and P-glycoprotein, showed that riboflavin was increased in the Bcrp−/− and the Bcrp and P-glycoprotein−/− mice but not the P-glycoprotein−/− mice, an indication that riboflavin is a BCRP substrate rather than a substrate of P-glycoprotein (Zhang et al., 2023). Riboflavin, also known as vitamin B2, is a water-soluble vitamin precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), critical cofactors for numerous redox metabolic reactions that control cellular homeostasis (Suwannasom et al., 2020). Like riboflavin, isobutyryl carnitine and arginine were among the increased metabolites in Bcrp−/− mice (Zhang et al., 2023), but contrary to what was observed for riboflavin, treatment with a BCRP and a P-glycoprotein inhibitor (elacridar) failed to increase the levels of isobutyryl carnitine and arginine, indicating that their increases in the metabolomics library were not relying solely on BCRP (Zhang et al., 2023). The identification of riboflavin as a preferred substrate for BCRP lays the foundations for the newly published work by Dr. Shen and colleagues (2024) in this number of JPET, who demonstrated in healthy individuals that riboflavin is a promising biomarker to monitor the inhibition of BCRP, which occurs after treatment with BMS-986371 (Zhang et al., 2023). Previously known for its covalent inhibition of mitogen-activated protein kinase–activated protein kinase 2, BMS-986371 has also shown anti-inflammatory effects and inhibitory effects toward BCRP (intestinal efflux transport protein) and toward hepatic transport proteins (Gaur et al., 2022; Malona et al., 2022). To test the suitability of riboflavin as a biomarker for uptake and efflux of drugs, the authors performed numerous experiments in the human embryonic

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ABBREVIATIONS: BCRP, breast cancer resistance protein; DDI, drug-drug interaction; MATE, multidrug and toxin extrusion; MTX, methotrexate; OAT, organic anion transporter; SSZ, sulfasalazine.

kidney cell line HEK-296 overexpressing the apical membrane transporters multidrug and toxin extrusion (MATE)1 and MATE2-K and the basolateral membrane transporters organic anion transporting polypeptide (OATP)1B1, OATP1B3, organic anion transporter (OAT)1, OAT3, and OCT2. The data showed that the uptake of riboflavin occurs preferentially via OAT1, OAT3, and MATE2-K. Additionally, the authors tested the inhibitory potential of BMS-986371 over BCRP and P-glycoprotein in the porcine kidney cell line LLC-PK1 and found that both BCRP and P-glycoprotein as well as hepatic transport proteins but not renal transport proteins were inhibited. Collectively, the experiments conducted in cultured cells provide convincing evidence that riboflavin could be a suitable biomarker to monitor BCRP activity. However, using more physiologically relevant cell lines that recapitulate the BCRP expression and activity such as intestinal and hepatic cell lines would increase the level of confidence toward these findings.

The in vitro experiments provide the basis for the next part of the study, focused on two phase I clinical trials in healthy individuals with the goal to determine the suitability of riboflavin as a reliable BCRP biomarker, predictive of an efficient BCRP inhibition after treatment with BMS-986371. In the first clinical trial (NCT04268394), the aim was to assess safety, tolerability, and pharmacokinetics of BMS-986371 given alone or in combination with methotrexate (MTX) and sulfasalazine (SSZ) (given as immediate-release formulation), known substrates of BCRP (Haagsma et al., 1996) typically administered in combination for rheumatic diseases. The results of this clinical trial showed that BMS-986371 administered at the same dose used in rheumatic diseases (150 mg) inhibited BCRP more efficiently than P-glycoprotein, thus establishing BCRP as a critical target to study transporter-mediated DDI. This result also underscores that despite the improvements of the prediction models used, the in vitro effects of BMS-986371 were not completely recapitulated in humans, as the BMS-986371 effects on P-glycoprotein were not as strong as in the experiments conducted in LLC-PK1. The aim of the second clinical trial (NCT05445440), also conducted in healthy individuals, was to determine the effects of BMS-986371 on MTX, on different formulations of SSZ (given as immediate release and enteric-coated formulations), and on the MTX-SSZ cotreatment. The authors reported small but consistent increases of riboflavin in the plasma of healthy individuals over the 2-week treatment duration and no significant increases in the levels of isobutyryl carnitine and arginine, two additional potential BCRP biomarkers included in the study, indicating that the BMS-986371 actions are specific for riboflavin. However, further considerations regarding riboflavin as a preferred BCRP biomarker need to be evaluated. One important aspect involves the high interindividual riboflavin fluctuations that may complicate the interpretation of the data among patients, in part because humans do not synthesize riboflavin; thus, the levels of this nutrient heavily rely on the diet and on the health of the intestinal walls. Prescribing a strict diet while patients are on BMS-986371 treatment could mitigate the interindividual fluctuations in the levels of riboflavin. Additional clinical trials assessing the potential of riboflavin should include cohorts with a prevalence of women, ethnically diverse cohorts, and cohorts exploring older individuals than the ones recruited in the current clinical trials. Being able to monitor riboflavin as a surrogate biomarker of BCRP using minimally invasive tests such as a blood draw has the potential to improve a wide spectrum of therapies, including those that include the administration of multiple drugs. Along with the promising proof-of-concept results that Shen and colleagues (2024) presented in the clinical trials, there are some aspects that will need to be addressed should BMS-986371 move forward as a candidate drug to inhibit BCRP. In addition to the interindividual variability of baseline levels of riboflavin also discussed by the authors, it will be key to further investigate the riboflavin absorption efficiency in individuals with intestinal disorders or in those who assume therapies that disrupt the intestinal walls, given that the BCRP expression is mainly in the intestine and that riboflavin is absorbed from the diet.

Although at an initial stage, this research could provide significant improvements in the pharmacokinetics of multiple drugs and offers an approach that can be applied to discover more DDI predictive molecules. Emerging strategies that are quickly revolutionizing drug discovery and development include artificial intelligence and machine learning (Kong et al., 2023) that can be used to confirm potential molecules found with the current predictive models, improve tissue specificity and species specificity of hundreds of proteins, and minimize the identification of false positives. A combination of computerized technologies and analytical methods for experimental validation will likely propel the field of drug discovery forward.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Melis.

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