TOXICOLOGICAL HIGHLIGHT

Metabolomics and Mechanisms: Sometimes the Fisher Catches a Big Fish

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Received September 17, 2010; accepted September 18, 2010

Key Words: metabolomics; metabonomics; ethylene glycol monomethyl ether; flavoprotein dehydrogenases.

In the history of science, natural phenomena are described, using progressively more refined techniques, until accumulated observations can be explained by unifying hypotheses. Metabolomics (also termed metabonomics) has been maturing greatly for more than 20 years (Gartland et al., 1989) and has expanded from studies describing the effects of selected compounds that perturb specific body systems and identifying biomarkers (Lindon et al., 2003) to studies of the broad effects on intestinal microbial flora (Jia et al., 2008; Robosky et al., 2005), a vast new frontier for biologists in which more than 1200 new species from the human intestine can be described in a single paper (Breitbart et al., 2003). Metabolomics has even become useful in epidemiological investigations (Bictash et al., 2010). Improved analytic techniques (Beckonert et al., 2010) and newly developed chemometrics methods (Cloarec et al., 2005; Jourdan et al., 2010) have contributed greatly to the enhanced capabilities of metabolomics. As impressive as these broad systems biology approaches are, metabolomics and the other -omics techniques have been subject to the disparaging slur, “It’s a fishing expedition.” As someone who spent a summer working on an Alaskan commercial salmon fishing boat, I have always been bothered by this metaphor as a way of condemning nonhypothesis–driven research. Accordingly, it is personally delightful to see metabolomics studies, combined with insightful comparative pathway analyses and a thoughtful assessment, put to use to develop innovative and testable hypotheses about mechanisms of actions of specific toxins.

In the highlighted article in this issue of Toxicological Sciences, Takei et al. (2010) used metabolomics to develop a new hypothesis for the mechanism of action of ethylene glycol monomethyl ether (EGME) toxicity. This solvent has been used in a variety of industrial processes, including production of cellulose acetate, resins, paints, inks, and stains and as antifreeze in jet fuels and hydraulic fluids. It causes toxicity in tissues with rapidly dividing cells and high metabolism such as testes and thymus. As is often the case, single-dose studies have described various ill effects induced by EGME but have not uncovered a clear mechanism of action. Accordingly, Takei et al. (2010) undertook a more comprehensive study, dosing for 14 days, with sacrifices at early, middle, and late time points, using two doses, a toxic dose that caused histological lesions and a low dose that did not cause histological lesions to show early biochemical changes. The endpoints were examined in multiple organs and body fluids using three independent, complementary, and broad-spectrum metabolomic platforms capable of identifying and differentiating hundreds or perhaps thousands of endogenous metabolites: ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) optimized for basic molecules, UHPLC/MS/MS optimized for acidic molecules, and gas chromatography/mass spectrometry (Evans et al., 2009).

As is often the case in complicated body systems where multiple tissues are affected by various processes with overlapping time trajectories, the authors found that hundreds of endogenous metabolites were perturbed in each organ or body fluid. At this point, the analysis got interesting. Using Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) to analyze which pathways were perturbed and focusing their attention on early time points, the authors identified alterations in three seemingly disparate major pathways—choline oxidation, branched-chain amino acid catabolism, and fatty acid β-oxidation. From the metabolites that were increased or decreased, the authors inferred which enzymes were inhibited.

For example, isoleucine has two pathways of catabolism, called the S-pathway, by which isoleucine produces succinyl-CoenzymeA (CoA) which is incorporated into the tricarboxylic acid cycle for energy, and the R-pathway, in which isoleucine is...
degraded to various metabolites. As early as 4 days in rats given either the subclinical or the clinical dose, there was an increase in 2-methylbutyrylglycine, 3-hydroxy-2-ethylpropionate, ethylmalonate, and butyrylglycine in the urine and butyrylcarnitine, 2-methylbutyrylcarnitine, and 3-hydroxy-2-ethylpropionate in serum, testes, and liver (Fig. 1). One explanation for this pattern is inhibition of short/branched-chain acyl-CoA dehydrogenase, the enzyme in the S-pathway that converts 2-methylbutyryl-CoA to tiglyl-CoA. Inhibition of this enzyme could cause an increase in its substrate, 2-methylbutyryl-CoA, leading to increased degradation to 2-methylbutyrylglycine and 2-methylbutyrylcarnitine. Additionally, inhibition of the S-pathway would increase diversion of isoleucine to the R-pathway. This is consistent with the increase in R-pathway metabolites, 3-hydroxy-2-ethylpropionate, butyrylglycine, butyrylcarnitine, and ethylmalonate. Using similar logic, the authors hypothetically identified the other enzymes that could explain the metabolite perturbations seen in the choline oxidation, other branched-chain amino acid catabolism, and fatty acid β-oxidation pathways—dimethylglycine dehydrogenase; sarcosine dehydrogenase; isovaleryl-CoA dehydrogenase; and the short-, medium-, long-, and very long-chain acyl dehydrogenases, among others.

The key mechanistic insight was the unifying observation that each of these enzymes was a primary flavoprotein dehydrogenase with a flavin adenine dinucleotide (FAD) prosthetic factor. This suggested the novel hypothesis that the mode of action of EGME-induced toxicity is inhibition of the flavoprotein dehydrogenase enzyme family. This hypothesis

FIG. 1. Isoleucine catabolism (from Takei et al., 2010). The box plots show isoleucine catabolites altered by EGME treatment. The box represents the middle 50% of the distribution, and the upper and lower ‘‘whiskers’’ represent the entire spread of the data. The hyphen refers to the mean. The y-axis represents the median-scaled value. SBCAD: short/branched-chain acyl-CoA dehydrogenase.
allowed the authors to query human genetic disorders with similar enzyme deficiencies and ask if they had similar metabolic signatures, thus strengthening their argument. There is a human genetic disorder called multiple acyl-CoA dehydrogenase deficiency (MADD), a disease characterized by impairment of primary flavoprotein dehydrogenase enzymes. A number of studies report a similar spectrum of changes in endogenous metabolites as found by Takei et al. (2010) in their EGME-dosed rats. However, the clinical presentation of MADD involves different organ systems, including heart, liver, and kidney (Freeman and Goodman, 2001), and this difference in target organs, along with some notable differences in the urinary and serum metabolites of MADD patients (Freeman and Goodman, 2001), remains a challenge for the hypothesis of EGME-induced flavoprotein dehydrogenase inhibition to explain.

The beauty of an insightful and unforeseen hypothesis is that it leads to more detailed mechanistic questions. Takei’s data do not allow them to determine how EGME inhibits the flavoprotein dehydrogenases. They speculate that EGME might disrupt specific steps in the cascade of the FAD-mediated electron transport chain to the ubiquinone pool in the inner mitochondrial membrane. This proposal would be amenable to in vitro testing. Alternatively, they propose a posttranslational modification mechanism, in which the flavoprotein dehydrogenases might be blocked by hyperacetylation, consistent with the observation that that methoxyacetic acid, the active metabolite of EGME, inhibits histone deacetylase activity and increases histone acetyltransferase activity, resulting in increased acetylation of core histones (Wade et al., 2008).

Whatever the ultimate mechanism of EGME action on the flavoprotein dehydrogenase enzymes, it is clear that the non-targeted metabolomic analysis of Takei et al. (2010) have taken our understanding of EGME toxicity from the phenomenological to the mechanistic. Sometimes, the fisher catches a big fish.

REFERENCES


