Drugs, bugs, and personalized medicine: Pharmacometabonomics enters the ring

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The development of personalized treatment regimens, optimized to the measured biological status of the patient, to maximize benefits and minimize adverse effects, represents a major goal for 21st-century medicine (1). It is axiomatic that not all individuals respond to drug treatment in the same way, with lack of efficacy and adverse drug reactions (particularly idiosyncratic toxicity) representing a major cause of concern for both clinicians and the pharmaceutical industry.

The reasons for the success or failure of any clinical intervention are many fold, with a subject's pathophenotype dictating the likely outcome. Clearly such phenotypes result from many variables with genetic makeup, physiological factors such as age, gender, stress, disease, etc., and environmental factors such as diet, lifestyle, exposure to environmental toxins and environmental history (including in utero experiences), concomitant drug and alcohol usage, and even, or perhaps especially in light of emerging experimental work in areas such as diabetes and obesity and gut microbiology (2, 3). Self-evidently, therefore, interindividual variation in response to therapy is strongly influenced by the patient's biochemical state at the time of treatment, as reflected by his metabolic phenotype, and this phenotype results from the interaction of both genetic background and environmental factors (4). Although the desire of physicians to treat their patients as individuals and provide personalized drug treatment is in no way new, the problem has always been how to do this?

Genotypes and Phenotypes

One area of great promise is pharmacogenomics, where attempts have been made to use our increasing knowledge of the human genome to more carefully target drugs so that the right medicine is given to the right patient (at the right dose). Pharmacometabonomics is a more recent approach that uses metabolic phenotypes to predict the metabolism or toxic effects of drugs (5). This concept is predicated on the idea that the subject's metabolic profile represents a phenotype in its own right [the metabotype (6)], resulting from the concatenation of many physiological, chemical, genetic, and environmental influences.

In this issue of PNAS, we now have a demonstration of this pharmacometabonomic approach in humans (7). The metabotype is both statistically and biologically related to intervention outcome probabilities (4) and, given that metabolites represent real outcomes, rather than the potential outcomes encoded in the genes, metabotypes may in fact provide one of the best links to the patient's pathophenotype. One of the major factors influencing a patient's response to any medication is drug metabolism. This term encompasses the ability to absorb, distribute in the body to the site of action, detoxify by metabolism, and then eliminate the drug (e.g., via urine or bile) (ADME). The ADME properties of a drug are governed by a range of drug transporters and metabolizing enzymes such as cytochrome (CYP) P450s, *N*-acetyl transferases, sulfotransferases, and glucuronosyl-transferases. Differences in the balance of metabolism leading to detoxification vs. toxicity are the difference between a treatment being safe and effective or causing an adverse drug reaction. Thus far, attempts at personalized approaches to predicting drug metabolism/toxicity have been based on genotypic variation and polymorphisms in drug-metabolizing enzymes. However, pharmacogenomic prediction of these has had limited success (8). To date, pharmacometabonomics has been demonstrated in rats, predicting xenobiotic toxicity and metabolism from predose urinary metabolite profiles (5). In the case of rats given acetaminophen, directly relevant to the article in this issue of PNAS (7), a significant association was found between metabolic fate, liver damage, and predose metabotype. The study in ref. 7 is important because it shows that pharmacometabonomics can be translated to humans receiving therapeutic doses of acetaminophen, with an individual's predose urinary metabolite profile able to predict the metabolic fate of the drug. These predose metabolite profiles showed that individuals excreting comparatively high concentrations of *p*-cresol-*O*-sulfate were prone to excrete relatively less acetaminophen-*O*-sulfate and larger amounts of acetaminiophen-*O*-glucuronide than people excreting low amounts of *p*-cresol-*O*-sulfate. Acetaminophen and *p*-cresol, as aromatic phenols, are structurally quite similar and both compete for sulfation.

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Microbiomes and Drug Metabolism

Interestingly, however, *p*-cresol is not derived from the metabolism of the person but is produced by gut-dwelling bacteria that form part of each individual's unique "microbiome." The microbiome provides a rich source of extragenomic interindividual variation in metabolic phenotype, and there is considerable evidence that microbiomes vary between populations and individuals (9, 10), with measurable consequences for drug ADME properties. So, interethnic variation in the reduction of the cardiac drug digoxin by the microbiota has been described (11, 12) with increased production of reduced metabolites in North Americans (36%) compared with a South Indian population (13.7%). Within the South Indian population differences were seen for rural vs. urban dwellers with only 5% of the former excreting reduced metabolites compared with 23% of the latter (12). Although reductive metabolism is important, the gut microbiota can perform a wide range of xenobiotic biotransformations including hydrolyses, decarboxylations, dehydroxylations, dealkylations, dehalogenations, and deaminations (13), providing the potential for a rich source of variability in host biochemistry and reac-

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tions to treatments quite unrelated to the host genome.

Examples of microbial effects on pharmacologically active natural products include effects on soy-derived phytoestrogens conducted in germ-free and "humanized" rats, colonized with human gut-derived microorganisms from several donors (14). Germ-free rats excreted large quantities of isoflavones in urine after administration of soy isoflavones, but the pharmacologically active isoflavone metabolites equol *O*-desmethylangolensin and the lignan enterolactone were absent. Rats colonized with human-derived microbiota did excrete these active metabolites but it was donor dependent and only seen for animals with bacteria derived from equol-producing human donors. Those humans unable to make equol, etc. also failed to induce equol production in rats. Thus, the microbiota were essential for the delivery of the pharmacologically active substances needed for efficacy. Such interindividual variability would clearly not be predicted by gene-centric personalized medicine approaches. These soy-derived phytoestrogens also affect host steroid metabolism, causing, among other things, a reduction in genotoxic total estrogen metabolite excretion with reduced excretion of 4-hydroxyestrogen and increased 2-hydroxyestrogen (15). Xu et al. (15) suggested that the soy products produced via the gut microbiota affected the CYP isoenzymes responsible for estrogen hydroxylation. There are numerous other instances of effects of microbiota dietary-derived compounds modulating xenobiotic metabolizing systems (13).

The effects of microbially derived *p*-cresol on the metabolism of acetaminophen in this new study (7) serve to remind us of a number of often forgotten facts. In particular, we should remember that drug-metabolizing enzymes evolved to deal with plant and microbial products and toxins, not drugs, and we can expect to find many other instances where modern drugs interact with the gut microbiome in unexpected ways. Such interactions can be via direct effects, as seen with digoxin, the induction of xenobiotic metabolizing systems (e.g., P450s), or competition for detoxification pathways. The bacterial-derived *p*-cresol will compete for sulfation with all phenolic drugs/metabolites, not only acetaminophen. This simple biomarker therefore may have pharmacometabonomic significance well beyond acetaminophen metabolism. Importantly the competition for limited "sulfur" resources will affect other pathways, such as those required for the production of compounds such as glutathione, which is intimately related to cellular defense against reactive electrophiles of the type generated by acetaminophen. Indeed, the lower production of *N*-acetylcysteinyl conjugates of acetaminophen seen when *p*-cresol sulfate excretion was high could be interpreted as a reduced capacity for detoxification of these reactive metabolites. It is easy to envisage circumstances where an individual with a diet low in sulfur-containing amino acids and a microbiome high in of *p*-cresol-producing microbiota might suffer an "idiosyncratic" toxic response to acetaminophen (or indeed any other drug that undergoes a similar sulfur-dependant detoxification process), whereas individuals on the same treatment regime with an adequate diet and low exposure to *p*-cresol do not. In addition, depriving the subject of essential sulfur-containing amino acids via xenobiotic detoxification also prevents their use in protein synthesis and other important sulfur-dependant anabolic processes. A consequence of this sequestration has been shown for the rat where daily oral administration of nontoxic doses of acetaminophen to juvenile animals prevented growth (16). However, understanding the cause of these effects allows rational interventions to be made and the gut microflora are an eminently "druggable" target (17). With the bacterial reduction of digoxin noted earlier, the administration of antibiotics (erythromycin or tetracycline) resulted in drug concentrations in serum rising 2-fold (18). If the production of *p*-cresol, and its depletion of sulfur, was a problem, then an alternative response to the use of antibacterials could be the administration of dietary supplements. In the case of rats suffering from arrested growth after acetaminophen administration addition of methionine to the diet resulted in normal growth.

The importance of this work (7) is that it provides a convincing demonstration of pharmacometabonomics in humans while at the same time revealing a hitherto-unrecognized specific effect of the gut bacteria on drug sulfation and detoxification. This work may well have important implications for individual patients via the improved delivery of personalized medicine studies, thus influencing the policies of healthcare providers and drug discovery and development.

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