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Stable isotopes, mass spectrometry, and molecular fluxes: Applications to toxicology

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Abstract

In order to meet the increasing demands for safe and affordable drugs, improvements in the efficiency and accuracy of every step in drug development are required. Accordingly, new approaches for assessing drug toxicity that are faster and more precise are in demand. Numerous approaches using -omics and systems biology are being developed to meet this demand and, while promising, they have not yet provided the improvements in toxicology promised. Other innovative methodologies for predicting and assessing toxicities should therefore be explored. Here we present a novel approach for directly measuring the in vivo response of specific metabolic pathways to toxic agents. Using stable isotopes and ultra sensitive mass spectrometry, the effect of an agent on myelin synthesis, protein synthesis, or cell proliferation can be directly measured. Examples are presented where this approach is used to detect toxicity in the liver, brain, peripheral neurons, breast, and skin. Collagen synthesis, microglia proliferation, myelin synthesis, tubulin synthesis, hepatic cell proliferation, epidermal cell proliferation and mammary epithelial cell proliferation are quantitatively determined in vivo, in a high throughput manner. This approach avoids the computationally complex approach of systems biology and allows the user to observe the emergent properties of the system directly and quantitatively.

Keywords: Deuterium; Stable isotope; Flux; Rat; Mouse; Methods; Neurotoxicity; Hepatotoxicity; Mitogen; Fibrosis

1. Introduction

In the post-genomic era it has become clear that a purely reductionist approach is inadequate to describe, let-alone predict, the complex behavior of biological systems, even relatively simple ones (Koffas & Stephanopoulos, 2005; Stephanopoulos, 1999; Stephanopoulos, Alper, & Moxley, 2004). The goal of systems biology is to describe the emergent properties of assembled biological systems (organisms) and ultimately translate that information into a form that can be used to diagnose or treat disease. Enormous advances have been made in the analytical and computations tools used for studying systems biology. The translation of these advances into usable pharmacological and toxicological tools has been slower to emerge, however. Alternative and complementary approaches to understanding complex biological networks should therefore be explored.

An alternative to a reductionist approach is to study fully assembled systems; an approach that has always been widely appreciated but has lacked tools that are analytically powerful enough to make mechanistic or predictive evaluations. Direct measurement of the metabolic activity or flux through critical biochemical pathways with stable isotopes may provide the analytical precision and quantification needed to make decisions about the pharmacology and toxicology of new chemical entities. In fact, stable and radioisotopes have been used by biochemists and physiologists to measure fluxes (the activities of metabolic pathways) in vivo for decades. These approaches have, until recently, been limited to academic labs however because of their relatively complex and low-throughput nature. Recent advances in mass spectrometry and new methods for labeling metabolic pathways in vivo have lead to the development of numerous new applications of stable isotopes in drug discovery

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(Hellerstein & Murphy, 2004; Turner & Hellerstein, 2005), medicine (Hellerstein, 2003, 2004) and toxicology.

As molecular and computational tools advance, our ability to test and validate the predictability of -omics based analyses will undoubtedly be the next technological hurdle. Methods which improve the observability of intact complex systems, such as described here, could not only validate -omics type signatures but serve as predictive biomarkers in their own right. By dramatically improving the detection of a phenotypic response, real insight about dose, exposure, and risk can be had.

This review focuses on applications developed in our laboratory which are likely to have applications in preclinical and environmental toxicology. Clinical and drug development applications have been reviewed elsewhere (Hellerstein & Murphy, 2004; Turner & Hellerstein, 2005). The general principles of measuring kinetics with stable isotopes, reviewed in (Wolfe, 1992; Dufner & Previs, 2003), remain consistent across these applications.

2. Kinetic biomarkers in physiology

Truly predictive toxicology will require radically different approaches that go beyond extrapolation from high dose rodent studies. Arguably these new tools must move away from merely observing or identifying biomarkers of pathological outcomes (histology, clinical chemistry) and move towards more sensitive measures of physiological changes. These changes may be early manifestations of direct toxic damage or may be effects that predispose or enhance the probability of a toxic response in an organism when combined with other environmental or genetic factors. Improved "observability" of key metabolic pathways involved in an organism's response to toxins would allow determination of precise phenotypic outcomes in vivo against which complete dose responses or sensitivity could be evaluated.

Stable isotope/mass spectrometric methods have been developed which dramatically improve in vivo observability and predictability of intact metabolic pathways over conventional physiological measures. Measuring the molecular flux, or kinetics, of a specific pathway in vivo gives the observer two unique and otherwise unobservable pieces of information about the system: 1) where it is going, and 2) how fast it is getting there. The ability to measure the kinetics of a biological system has several important practical features that distinguish kinetic measurements from static ones, such as protein or metabolite concentration or composition.

First, changes in flux through any pathway must change before there can be a change in the concentration of the pathway's product. For example, flux though collagen synthesis or degradation pathways must change before there can be the accumulation of excess collagen which then leads to fibrosis. The practical implication is that measuring the change in kinetics can detect, and indeed predict, the development of slowly progressing diseases, such as demyelination in the brain or peripheral nervous system or lung and hepatic fibrosis (see below).

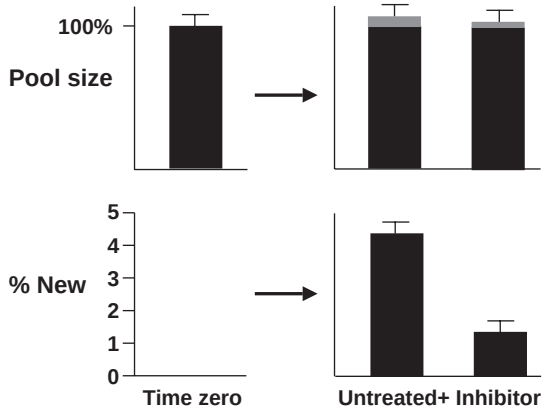

Fig. 1. An illustration of the difference between a measurement of the change in pool size (top panel) and the kinetic measurement of the difference in fractional synthesis.

Second, kinetic measurements can detect much smaller changes than static markers. Because isotope approaches measure only what is new over the study period, the "noise" of the pre-existing pool of molecules or cells is effectively eliminated from the measurement. This is graphically illustrated in Fig. 1. This can be of particular importance when evaluating large pool-size, slow turnover systems or systems which have feedback regulatory components such that a perturbation in pool size is significantly compensated for by an opposing pathway.

Finally, kinetic biomarker measurements are made over days or weeks, thereby integrating any effects that occur in that time. This allows the kinetic biomarker to capture all diurnal and temporal fluctuations in response that can often confound and complicate interpretation of conventional biomarker analysis.

3. Kinetic biomarkers in toxicology

Our general aim is to develop kinetic biomarkers that are 1) an integral component of the pathological process and, therefore, unlikely to be dissociable from hard outcomes, 2) appear sooner than conventional measurements, and 3) are more sensitive at low exposures than conventional measurements. Six kinetic biomarkers are presented here which have shown promise as biomarkers of toxicity. As with any biomarker, extensive evaluation of sensitivity, specificity, and predictive ability are ultimately required. These biomarkers show extraordinary promise, however, because they measure a central component of a pathological response related to a toxicity and are therefore less likely to be confounded by secondary variables.

Each kinetic biomarker is presented individually with appropriate contextual background and one or more