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Detection of hepatotoxicity potential with metabolite profiling (metabolomics) of rat plasma

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ABSTRACT

While conventional parameters used to detect hepatotoxicity in drug safety assessment studies are generally informative, the need remains for parameters that can detect the potential for hepatotoxicity at lower doses and/or at earlier time points. Previous work has shown that metabolite profiling (metabonomics/metabolomics) can detect signals of potential hepatotoxicity in rats treated with doxorubicin at doses that do not elicit hepatotoxicity as monitored with conventional parameters. The current study extended this observation to the question of whether such signals could be detected in rats treated with compounds that can elicit hepatotoxicity in humans (i.e., drug-induced liver injury, DILI) but have not been reported to do so in rats. Nine compounds were selected on the basis of their known DILI potential, with six other compounds chosen as negative for DILI potential. A database of rat plasma metabolite profiles, MetaMap® Tox (developed by metanomics GmbH and BASF SE) was used for both metabolite profiles and mode of action (MoA) metabolite signatures for a number of known toxicities. Eight of the nine compounds with DILI potential elicited metabolite profiles that matched with MoA patterns of various rat liver toxicities, including cholestasis, oxidative stress, acetaminophen-type toxicity and peroxisome proliferation. By contrast, only one of the six non-DILI compounds showed a weak match with rat liver toxicity. These results suggest that metabolite profiling may indeed have promise to detect signals of hepatotoxicity in rats treated with compounds having DILI potential.

1. Introduction

The clear goal of safety evaluation studies is to characterize the hazards posed by a novel chemical. This goal (hazard characterization) is well established for human and environmental risk assessment (Faustman and Omenn, 2008) and drug development (ICH Steering Committee, 2009). Because overt toxicity is preceded by a complex sequence of biochemical, cellular and physiological events (Gregus, 2008), exposures to novel chemicals are evaluated with a variety of parameters that monitor these events (Baldrick, 2008; Crissman et al., 2004; ICH Steering Committee, 2009; Weingand et al., 1996). Furthermore, multiple parameters need to be considered to evaluate the sequence of events leading up to any given organ toxicity. For example, increases in the blood levels of alanine aminotransferase (ALT) may presage clear liver histopathology and failure (Ennulat et al., 2010; Senior, 2009; Travlos et al., 1996).

An organ toxicity that remains of critical interest to drug development is that of hepatotoxicity (Corsini et al., 2012; Horner et al., 2013). Current preclinical safety assessment study designs and parameters are effective for identifying a large number of chemicals with hepatotoxic potential, yet examples still exist of drugs that reach the marketplace only to then elicit cases of drug-induced liver injury (DILI), i.e., hepatotoxicity (Peters, 2005).

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It is well understood that hepatotoxicity may have several different pathological expressions, due to different mechanisms (Jaeschke et al., 2002; Russmann et al., 2009), and the molecular events in microcystin (Campos and Vasconcelos, 2010) and acetaminophen (Jaeschke et al., 2012) hepatotoxicity point to the complexity in the sequence of events leading to liver toxicity. Accordingly, research continues to seek new parameters that will monitor early events in hepatotoxicity to aid in liver safety assessment (Antoine et al., 2009).

New discoveries and technologies often offer new parameters that may complement those conventionally used in existing testing strategies. Ideally such new parameters fill gaps in evaluating the multiple events leading to toxicity, particularly in the ability to monitor early events in the sequence leading up to overt toxicity. Such parameters would be expected to provide signals at lower doses and/or earlier time points than provided by conventional parameters (that may be monitoring events later in the sequence). As an example, Kim 1 was discovered as a protein detectable in urine following kidney injury and has been recognized as detecting nascent nephrotoxicity undetectable by conventional clinical pathology (Vaidya et al., 2010). Such signals from the novel parameters in essence "predict" the signals (i.e., the toxicity) detected "later" by conventional parameters.

1.1. Metabolite profiling

One promising new technology that offers new parameters is that of metabolite profiling (metabonomics/metabolomics), the measurement in biological systems of the full complement of endogenous low-molecular-weight metabolites and their intermediates. Several technologies allow such a measurement from urine, plasma, or tissue extracts. This can offer a global view of the comprehensive metabolic response of a biological system to genetic or environmental modification (Clarke and Haselden, 2008). If such metabolic responses are occurring early in the sequence of events leading up to overt toxicity (Fig. 1), they could be used to detect the adverse potential of chemicals at an early stage in their development (Beger et al., 2010; van Ravenzwaay et al., 2012). Indeed, urine metabolites have been shown to detect cardiotoxicity, hepatotoxicity and nephrotoxicity induced by doxorubicin treatment at lower doses and earlier time points than conventional parameters (Wang et al., 2009). Similarly, urine

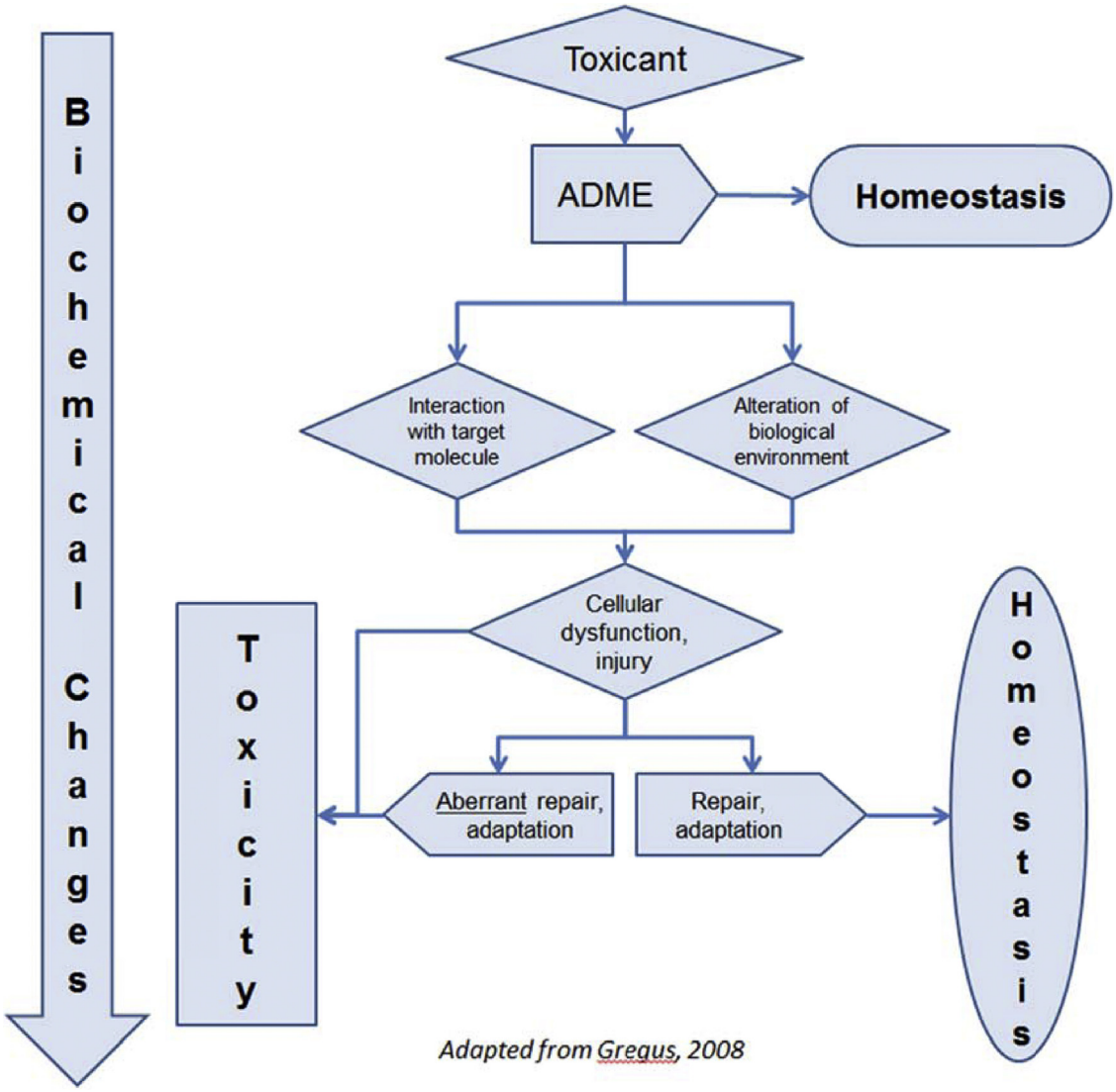

Fig. 1. An overview of the sequence of events following toxicant exposure.
Unique biochemical changes, reflected in metabolite profiles, may be expected at multiple stages in the sequence of events following toxicant exposure. Adapted from (Gregus, 2008).