Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 204 (2005) 135–151

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Comparative metabonomics of differential hydrazine toxicity in the rat and mouse

Mary E. Bollard[a,*], Hector C. Keun[a], Olaf Beckonert[a], Tim M.D. Ebbels[a,1], Henrik Antti[b,2], Andrew W. Nicholls[b,3], John P. Shockcor[b,4], Glenn H. Cantor[c], Greg Stevens[d], John C. Lindon[a], Elaine Holmes[a], Jeremy K. Nicholson[a]

[a]*Biological Chemistry, Biomedical Sciences Division, Sir Alexander Fleming Building, Imperial College London, South Kensington, London SW7 2AZ, UK*
[b]*Metabometrix Ltd., RSM, Prince Consort Road, London SW7 2BP, UK*
[c]*Bristol-Myers Squibb Company, Princeton, NJ 08543-5400, USA*
[d]*Pfizer Inc., La Jolla, CA 92037, USA*

Received 19 March 2004; accepted 21 June 2004
Available online 21 November 2004

Abstract

Interspecies variation between rats and mice has been studied for hydrazine toxicity using a novel metabonomics approach. Hydrazine hydrochloride was administered to male Sprague–Dawley rats (30 mg/kg, $n = 10$ and 90 mg/kg, $n = 10$) and male B6C3F mice (100 mg/kg, $n = 8$ and 250 mg/kg, $n = 8$) by oral gavage. In each species, the high dose was selected to produce the major histopathologic effect, hepatocellular lipid accumulation. Urine samples were collected at sequential time points up to 168 h post dose and analyzed by ^{1}H NMR spectroscopy. The metabolites of hydrazine, namely diacetyl hydrazine and 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid (THOPC), were detected in both the rat and mouse urine samples. Monoacetyl hydrazine was detected only in urine samples from the rat and its absence in the urine of the mouse was attributed to a higher activity of *N*-acetyl transferases in the mouse compared with the rat. Differential metabolic effects observed between the two species included elevated urinary β-alanine, 3-D-hydroxybutyrate, citrulline, *N*-acetylcitrulline, and reduced trimethylamine-*N*-oxide excretion unique to the rat. Metabolic principal component (PC) trajectories highlighted the greater degree of toxic response in the rat. A data scaling method, scaled to maximum aligned and reduced trajectories (SMART) analysis, was used to remove the differences between the metabolic starting positions of the rat and mouse and varying magnitudes of effect, to facilitate comparison of the response geometries between the rat and mouse. Mice followed "biphasic" open PC trajectories, with incomplete recovery 7 days after dosing, whereas rats followed closed "hairpin" time profiles, indicating functional reversibility. The greater magnitude of metabolic effects observed in the rat was supported by the more pronounced effect on liver pathology in the rat when compared with the mouse.

Keywords: Metabonomic analysis; Hydrazine toxicity; Rat; Mouse

Abbreviations: 2-AA, 2-aminoadipate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEST, Bruker efficient sample transfer; DMA, dimethylamine; DMG, dimethylglycine; LC-MS, high performance liquid chromatography coupled to mass spectrometry; MA, methylamine; NMR, nuclear magnetic resonance; LC-NMR-MS, liquid chromatography coupled to nuclear magnetic resonance and mass spectrometry; PC, principal component; PCA, principal component analysis; PLP, pyridoxal 5′-phosphate; SD, Sprague–Dawley; SMART, scaled to maximum aligned and reduced trajectories; TCA, tricarboxylic acid; THOPC, 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid; TMA, trimethylamine; TMAO, trimethylamine-*N*-oxide; TSP, 3-trimethylsilyl-1-[2,2,3,3,-$^2H_4$] propionate.

* Corresponding author. Biological Chemistry, Biomedical Sciences Division, Sir Alexander Fleming Building, Imperial College London, Exhibition Road, South Kensington, London SW7 2AZ, UK. Fax: +44 20 7594 3226.
E-mail address: mary.bollard@imperial.ac.uk (M.E. Bollard).
[1] Present address: Bioinformatics Unit, Department of Computer Science, UCL, Gower Street, London WC1E 6BT, UK.
[2] Present address: Department of Chemistry, Umeå University, 90187 Umeå, Sweden.
[3] Present address: Investigative Preclinical Toxicology, Glaxo SmithKline Pharmaceuticals, Park Road, Ware, Herts SG12 0DP, UK.
[4] Present address: Bruker BioSpin Corporation, 15 Fortune Drive, Manning Park, Billerica, MA 01821-3991, USA.

0041-008X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2004.06.031

Introduction

Understanding species differences in toxicity to drugs is of fundamental importance in assessing mechanisms of drug-induced toxicity as well as being crucial to understanding risk assessment of new chemical entities within pharmaceutical development. Recently, new methodologies have been developed to investigate the holistic responses of animals to toxic and disease processes, through the use of advanced analytical techniques, such as nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography coupled to mass spectrometry (LC-MS), and liquid chromatography coupled to NMR and MS (LC-NMR-MS) together with multivariate statistics (termed metabonomics) (Nicholson et al., 1999, 2002). An advantage of the metabonomic approach is that time course changes in individual animals can be followed during the onset, progression, and potential recovery of lesions (Beckwith-Hall et al., 1998; Holmes et al., 1992, 1998a, 2000; Robertson et al., 2000). The metabonomic approach results in metabolic trajectories that have characteristic geometries and specific directional changes that reflect key time points for pathological change. Here we show the first application of metabonomics to investigate species differences in toxicity using hydrazine as a model hepatotoxin.

Perturbations in the biochemical composition of biofluids reflect the metabolic adjustments that organisms make in order to maintain constancy of internal environment (homeostasis) (Nicholson and Wilson, 1989). ^{1}H NMR spectra of biofluids are highly complex, containing signals from potentially hundreds or thousands of low molecular weight metabolites (Nicholson et al., 1999, 2002). The efficiency of spectral interpretation can be enhanced by the use of pattern recognition and chemometric analysis (Anthony et al., 1994; Gartland et al., 1991; Holmes et al., 1998a, 1998b, 2000). Standard chemometric techniques, such as principal component analysis (PCA), can be used to map multidimensional spectral data of biofluids into a lower dimensionality, for visualization, where each sample occupies a position based on its metabolite composition. Therefore, samples that have similar biochemical composition, for instance two control samples, should occupy similar positions in the PC map. Toxicological lesions can be characterized by a sequence of events and the time course can be visualized by principal component (PC) trajectories (Holmes et al., 1992) that reflect the evolution of the lesion. The direction of the trajectory vector is directly related to the change in the endogenous urinary analytes excreted during the development of the toxic lesion and the magnitude of that vector movement reflects the severity of the lesion (Beckwith-Hall et al., 1998; Holmes et al., 1998a, 2000; Robertson et al., 2002). Metabolic trajectories also contain embedded mechanistic information (Nicholson et al., 2002). Although PCA is a useful visualization tool, it is not the most efficient means of comparing more than one model with very different magnitudes of response over time, for instance, from different species of animal. In this study, a recently developed SMART (scaled to maximum aligned and reduced trajectories) metabonomic approach (Keun et al., 2004) was used to both scale the data by the maximum response to hydrazine treatment in the rat and mouse and to remove pre-dose differences in metabolic mapping between species. This approach enables direct comparison of the direction of response of different species, despite their dissimilar magnitudes of response and so may be a valuable new tool for studying comparative species toxicity.

Hydrazine, a model hepatotoxin, was selected because it exerts species-dependent biochemical differences in toxicity that are poorly understood (Amenta and Johnston, 1962; Waterfield et al., 1993a). Hydrazine is also a metabolite of a number of pharmaceuticals, such as the anti-tuberculosis drug isoniazid and the anti-hypertensive drug hydralazine (Blair et al., 1985). The toxicity of hydrazine has been studied extensively in the rat, including its hepatotoxicity, carcinogenicity, mutagenicity, and neurotoxicity (Moloney and Prough, 1983; Sax, 1990). In one such study, hydrazine was found to be weakly carcinogenic in the Han Wistar rat when the compound was administered at 50 mg/L in drinking water, but not in the mouse (Steinhoff and Mohr, 1988). Biochemical responses of the rat to hydrazine as determined by urinary ^{1}H NMR spectroscopic studies have identified a range of urinary biochemical effects, including elevated β-alanine, taurine, creatine, and 2-aminoadipate (2-AA), together with decreased levels of glucose and 2-oxoglutarate (Holmes et al., 1998b; Nicholls et al., 2001; Sanins et al., 1990, 1992). Histopathological alterations in rat liver 24 h after the administration of hydrazine included decreased hepatic glycogen, with accumulation of fat in the periportal and mid-zonal regions of the liver (Amenta and Johnston, 1962). In contrast to the rat, the toxicity of hydrazine in the mouse is less well documented.

It is well known that metabolism and toxicological responses to xenobiotics may vary between species (Holmes et al., 1995, 1996; Johnson et al., 2000; Zbinden, 1993). Relatively little data have been published on the use of metabonomic techniques in laboratory species other than the rat, but the comparison to other species is an immediate issue. Screening of drug-induced toxicity is imperative within the pharmaceutical industry (Johnson and Wolfgang, 2001; Lakkis et al., 2002) and the mouse is an ideal model given its small size, requiring less test article than the rat and its extensive use in transgenic models of human disease (Boelsterli, 2003; Eynon and Flavell, 1999).

Here we investigate the differential metabolic effect and metabolism of hydrazine in the rat and mouse using NMR-based metabonomics, including SMART analysis.