INTRODUCTION

The identification of metabolites, whether for in vitro or in vivo samples, is an ongoing challenge for drug discovery and development. Metabolite identification typically uses an array of chromatographic and mass spectrometric methods, and therefore may require multiple injections of the same sample. This is to ensure that enough information has been collected to detect all metabolites and have fragmentation patterns available to elucidate structures. We describe a new workflow that enables the collection of both parent and fragment information from a single injection (Figure 1). Data acquisition used two scan functions interleaved such that the first scan function collected information about the intact metabolites and the second scan function collected fragment ions. With this approach, the entire data set is then re-used prior to post-acquisition processing for massive metabolite screening. To confirm the presence of a reactive intermediate, we used this approach by using an incubation of human liver microsomes in the presence of GSH for Nefazodone.

METHODS

Samples

Nefazodone was incubated with human liver microsomes at 10 μM at 37°C in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing the appropriate co-factors. GSH was added at a concentration of 10mM to the microsomal incubation. The reaction was terminated after 90 minutes with 2 volumes of cold acetonitrile to 1 volume of sample. Then, the sample was centrifuged at 13,000 rpm for 15 minutes and the supernatant was diluted 1/2 with Water +0.1 % formic acid. Mass analysis was accomplished through exact mass measurement. A variety of data processing algorithms can be used to extract metabolite information from these data. The data is acquired using high resolution (10K FWHM) and with mass accuracies typically in the sub-3 ppm range. The advantage of this approach is that, since all the data is collected in one run, post-acquisition processing for multiple product ions is possible. Neutral loss chromatograms may also be generated from the data using exact mass differences between the precursor and fragment ions. Since the data are acquired with no preconcentrations on the likely routes of metabolism, this approach has the potential to be truly comprehensive and universal in its use for in vitro reactive metabolism screening. From a single injection it is possible to detect all metabolites and have fragmentation patterns available for metabolite mining and very helpful to rationalize the putative biotransformations.

Background on Nefazodone

> Nefazodone is an antidepressant which was approved in the USA in late 1994

> In spite of its therapeutic effects there has been a number of cases (55 cases of liver failure (20 fatal) and another 39 cases of less severe liver failure) reported showing hepatotoxicity dysfunction and cholestasis (ref: Kalkutkar et al OWD 23:243-253, 2005).

LC-MS Methodology

LC/MS/MS DATA INDEPENDENT STRATEGY DETECTION AND IDENTIFICATION USING AN HTS METHODOLOGY FOR REACTIVE METABOLITE

Authors: Amy Bartlett!, Emma Marsden-Edwards1, Jose Castro-Perez2, Kate Yu2, John Shockcor2

Supernatant was diluted 1/2 with Water +0.1 % formic acid adjusted to pH 7.4 containing the appropriate co-factors. GSH 'Trapping' 1,2

MS-conditions:

LC-MS Methodology

• Lock Mass: Leucine Enkephalin at 200pg/mL

DMD 33:243-253, 2005

hepatobiliary dysfunction and cholestasis (ref: Kalkutkar et al OWD 23:243-253, 2005) ¾

cases (55 causes of liver failure (20 fatal) and another 39 USA in late 1994 ¾

The use of the TriWave device was beneficial as it was possible to conduct fractionation in two regions (Trap and Transfer) in a parallel fashion (Figure 3). This enhances the fragmentation coverage throughout the mass range acquired.

RESULTS

The data obtained by this approach was processed with MetaboLynx and 3 GSH adducts were detected m/z 789 (+O +GSH) (Figure 4).

Figure 1. Workflow showing the strategy for reactive metabo- lism screening

Figure 2. Schematic showing the data independent methodology for reactive metabolite screening

Figure 3. Mass loss monitoring with the loss of the pyroglutamic acid moiety

Figure 4. MetaboLynx browser report showing all 3 GSH adducts as well as other diagnostic fragment ions

Figure 5. Negative ion fragment information from m/z 789 corresponding to (+O+GSH) adduct of Nefazodone

• Not only GSH information was available with this approach but also other metabolite data

• From this strategy we were able to extract the low and high energy information for all metabolites and utilize the exact mass information from the low energy to remove false positives and confirm metabolites expected and unexpected

• The high energy information was used to obtain fragment ion and precursor ion information for structure elucidation (Figure 6 and 7)

CONCLUSION

Demonstrated a holistic approach to metabolite id and specific search for GSH conjugations

We can carry out as many NL or precursor ion experiments as required, we have access to 'all the data all the time'

One single injection provides information equivalent of multiple injections when compared to data dependent experiments and tandem quadrupoles or ion traps

Software tools and exact mass are very important to data mining and very helpful to rationalize the putative metabolites