“Structural Alerts in Reactive Metabolites (RM): a GSH Trapping Approach ”

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Introduction

The formation of reactive electrophilic metabolites was associated as potential mediator of cellular toxicity in early drug discovery. The mechanism involves covalent binding of the reactive metabolites (RM) to cellular proteins and then causing idiosyncratic drug toxicity. In order to trap any kind of reactive electrophilic metabolite, glutathione (GSH) in the media was used as the trapping agent to perform in-vitro metabolism experiments.

Analysis of “structural alerts” is a common practice in drug discovery. Possible functional groups that could be associates with RM formation are essential to understand and to mitigate the risk of hepatotoxicity in drug design, especially at the lead optimization and candidate selection phase. High Resolution Mass Spectrometry (HRMS) is the most powerful technique currently has capability to analyze the samples effectively and typically used in GSH trapping studies.

The purpose of this poster is to show the software assisted metabolite identification processes with a new automatic workflow for GSH-data analysis by using the HRMS. Structural alert analysis of GSH-adducts which identifies the chemical motifs that may cost the electrophilic attack was performed and presented in this study.

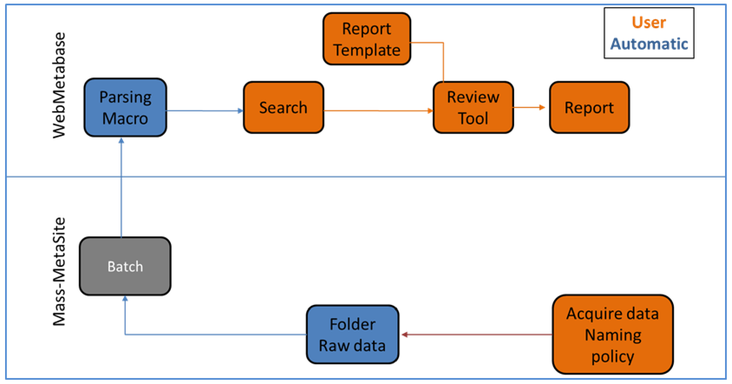
Method

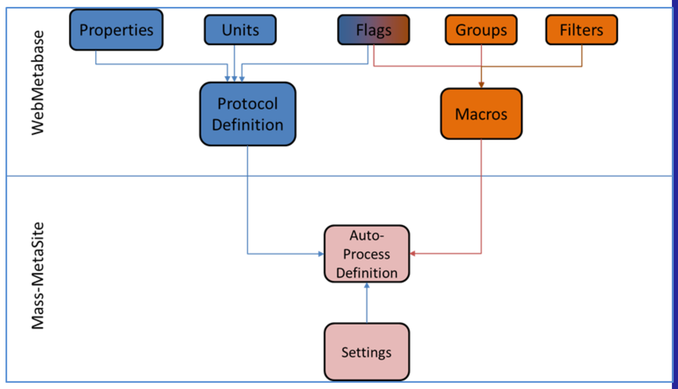
Human Liver Microsomes (HLM) at concentration of 1 mg/ml were incubated with 20 µM drug substrates in 0.1M sodium-phosphate buffer substituted with 1mM NADPH and 5mM GSH. After 60 minutes incubation, reaction was stopped by adding one volume acetonitrile.

Samples were analyzed by Waters UPLC-system coupled ABSciexTripleTOF™5600 mass spectrometer. Generic, targeted LC-HRMS method was applied to a set of 95 drugs. 21 structural alerts fragments were extracted from literature and evaluated for the fragmentational analysis (Kalgutkar, 2011).

Data analysis was performed with MassMetaSite and WebMetabase. After detection of chromatographic peaks, potential GSH adducts were identified based on GSH-related fragment ions and neutral-losses. Structural elucidation for each GSH-adducts was performed. Data (chromatograms, spectra, GSH adduct structures) was assembled in a searchable-database, WebMetabase, which was used for the comparison of GSH associated metabolic pathways for large compound sets, for the analysis of structure-activity relationships and also for the identification of common chemical motifs in GSH adduct formation.

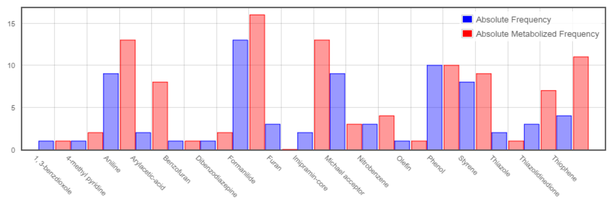
Workflow





Results

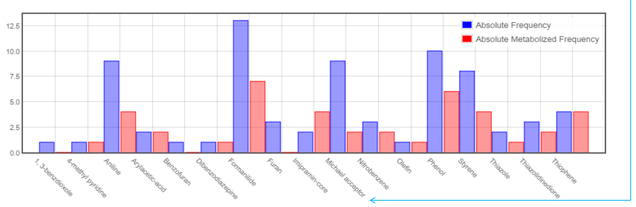
Reaction frequency on structural alerts



The blue columns are the number of times the structural alert fragment was found. Herein, the fragments were found 72 times in total.

The red columns display the number of all different reactions from each experiment that metabolized the found fragment (out of the blue columns). In this graphic, 103 reactions were counted in total.

GSH-conjugation reaction frequencies on structural alerts

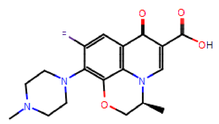


The blue columns are the number of times the structural alert fragment was found. Herein, the fragments were found 72 times in total.

The red columns are representing the number of different GSH-conjugation reactions from each experiment that metabolized the found fragments. In this graphic 41 reactions were counted in total.

In the plot of GSH-conjugation reaction frequencies some fragments showed the following results.
For instance:
Aniline was metabolized 4 times out of 9,
Formanilide was metabolized 7 times out of 13,
Phenol was metabolized 6 times out of 10,
Thiophene was metabolized 4 times out of 4.

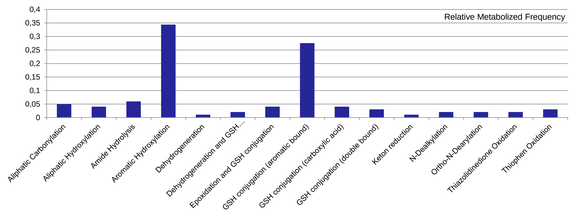
However, there are some cases where structural alert fragment is not always conjugated to GSH. E.g. Michael acceptor was found 9 times as a structural alert fragment, but it was metabolized 2 times by GSH-conjugation reactions. In the case of Levofloxacin, which is a safe categorized compound, there is not reported GSH conjugation in literature (Kalgutkar, 2011 & Nakayama, 2009). That means, a structural alert doesn’t have to be always metabolized by GSH-conjugation reactions.



Levofloxacin

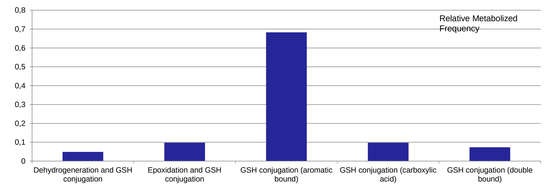
All reaction types found in structural alerts

Reaction mechanism relative frequencies extracted from the reaction frequency analysis

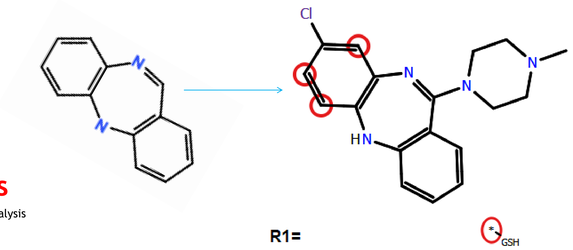


GSH-related reactions found in structural alerts

Reaction mechanism relative frequencies extracted from the GSH-conjugation reaction frequency analysis



A case study: Clozapine GSH adduct verification with the structural alert fragment of Dibenzodiazepine



The structural alert study was verified with fragment analysis. E.g. in Clozapine, the structural alert is dibenzodiazepine. According to the structural assignment results of MassMetaSite, the possible GSH adduct formation was marked with a markush system. The fragment analysis of Clozapine’s MS/MS spectra is supporting that the potential GSH nucleophilic attack would be in the structural alert part (dibenzodiazepine) of the drug molecule.

Conclusions

The structural alerts approach in the GSH trapping assay to detect the reactive metabolites (RM) by the software aided workflow is presented in this study. Structural information was extracted to point out which chemical motifs are more prompt to produce the GSH adducts and therefore more related to the hepatotoxicity. We applied a new workflow by MassMetaSite-WebMetabase combination to process a dataset out of the HRMS data quickly, to identify the drug-related GSH adducts semi-automatically and to obtain the fragment analysis of these GSH adducts within the structural alerts access automatically. According to our findings, we have observed that the common structural alerts were mainly metabolized by the GSH-conjugation reactions in agreement with the literature (Kalgutkar, 2011). However, it doesn’t mean that a structural alert will be always metabolized by GSH-related reactions. Sometimes, the safe categorized compounds could have structural alerts and they don’t involve with GSH-conjugations (Nakayama, 2009). In conclusion, structural elucidation and fragment frequency analysis of glutathione (GSH) conjugates may contribute to the improvement of the drug candidates by avoiding the certain structural scaffold prone to undergo metabolic bioactivation.

Acknowledgements

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References:

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Nakayama, 2009 “A Zone Classification System for Risk Assessment of Idiosyncratic Drug Toxicity Using Daily Dose and Covalent Binding” Pharmacology 37 (9): 1970-1977.