

Application Of Toxicity And Biomarker Workflows Within Ingenuity Pathways Analysis (IPA) Results In Efficient Evaluation Of Compound Toxicity And Safety

Brigitte Ganter¹, Dana L. Abramovitz¹, Adam S. Corner², ¹Ingenuity Systems, 1700 Seaport Blvd, 3rd floor, Redwood City, CA 94063; ²PO Box 776, Hampton Hargate, Peterborough PE7 8BB

Abstract

Ingenuity Pathways Analysis (IPA) is a software application that enables researchers to identify functions and pathways most relevant to experimental datasets or genes of interest and to understand the molecular mechanisms that underlie disease and cellular processes. We have integrated and curated information for over 5,000 exogenous and endogenous chemicals, toxicity relevant gene and pathway information, and a critical mass of relationships between those chemicals and gene products. Data analysis in the context of this chemical content as well as newly curated biomarker content, specifically, molecules detectable in different bodily fluids, such as urine, saliva, serum, plasma, and the suggested sentinel tissue blood, allows for quick identification of potential toxicity-biomarker candidates for candidate drug dose-time treatments under investigation.

We analyzed the liver expression profiles of 3 anti-proliferative compounds, carmustine, methotrexate, and thioguanine, from short term repeat dose studies in rats (Ganter *et al.* 2005). We were able to identify a discriminating gene set associated with carmustine's mechanism of toxicity, which was not found to be associated with the other two drugs, methotrexate and thioguanine. The newly added gene and compound relationship content in IPA further helped explain carmustine's observed liver toxicity, in particular bile duct hyperplasia. In conclusion, the toxicity and biomarker capabilities in IPA allow for efficient evaluation of compound toxicity and safety, which can be useful for compound ranking and prioritization earlier in the drug discovery and development process.

Background

Molecular toxicology employs genome-wide expression analysis linked to clinical pathology endpoints, an approach that offers distinct advantages over traditional toxicology technologies. Molecular toxicology is more sensitive in predicting or diagnosing clinical pathology endpoints associated with the compound or chemical series under investigation, and provides mechanistic understanding of the induced toxicological response that eventually leads to the observed clinical pathology.

To understand the mechanisms leading to toxicity by carmustine, the gene expression profiles of livers from rats treated at two doses (low and high dose) and three time points (1, 3, and 5 day) from short term repeat-dose studies for the three compounds were analyzed and compared using IPA. The toxicity of these compounds has been well characterized using traditional toxicology methodologies. All three drugs are toxic to the hematopoietic progenitor cells of the bone marrow and cause leukocyte depletion, whereas liver toxicity, marked by AST and ALT increase and observed bile duct hyperplasia, was observed primarily for carmustine treatments only (Ganter *et al.*, 2005). With this case study we demonstrate the power of IPA-Tox in elucidating the mechanisms underlying carmustine-induced hepatotoxicity from drug-induced gene expression changes.

References
Ganter, B., Tugendreich, S., et al., 2005, J. of Biotechnology 119 (3), 219-44.

Carmustine's Summary Quickly Reveals Toxicity to the Liver

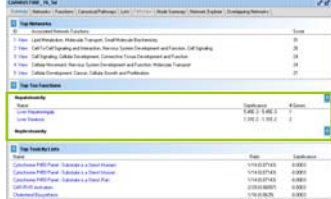


Figure 1: Automated and focused summary output of top safety data for carmustine reveals toxicity to the liver, such as hepatomegaly and steatosis.

Perturbation of CAR/PXR Activation and Hepatic Cholestasis Gene Lists



Figure 3: Carmustine's Toxicity Gene List analysis returned lists associated with xenobiotic metabolism and hepatic cholestasis. Toxicity lists are composed of functional gene groupings based on critical biological processes, such as key adaptive, defensive, or reparative responses to xenobiotic insult.

Functional Analysis Reveals Mode of Action and Toxicity

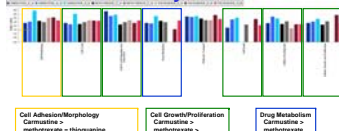


Figure 5: Carmustine's Biological Functions analysis returns cell adhesion and morphology, cell growth and proliferation, and drug metabolism functions.

Potential for Carmustine to Induce Hepatomegaly and Steatosis

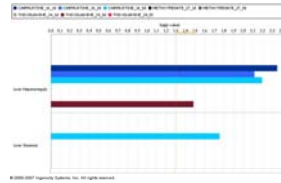


Figure 2: Functional Toxicity analysis for the 8 high dose drug treatments (all 3 anti-cancer drugs) reveals that the toxicity to the liver is specific for carmustine treatments with the exception of one high dose thioguanine treatment.

Induction of all Phases of Xenobiotic Metabolism



Figure 4: The impact of Carmustine HI 5day treatment on the different Tox Gene Lists reveals induction of xenobiotic metabolism. Visualized using the directional chart analysis within IPA 5.0

Carmustine Induces Immune Response and Cell Adhesion Genes

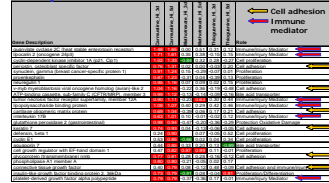


Figure 6: Shown are the most significantly induced genes from carmustine high dose treatments. Table exported from IPA.

Down-regulated Gene Set Associated with Mode of Toxicity



Figure 7: Shown are the most significantly down-regulated genes by carmustine treatments.

Network Analysis Reveals Mode of Action and Toxicity



Figure 9: Carmustine's Network analysis reveals mode of action. Molecular interaction networks are computationally generated from relationships stored in the Ingenuity Pathways Knowledge Base and molecular connections to the genes perturbed by carmustine treatment.

Summary

Ingenuity Pathways Analysis 5.0 and the IPA-Tox™ workflow revealed:

- Carmustine's discriminating gene sets (vs. methotrexate and thioguanine)
 - Up-regulated genes are enriched for molecules that are known to be associated with bile duct hyperplasia, such as cell adhesion, morphology, fibrosis, immune and injury modulators, and several transporters
- Carmustine induces hepatotoxicity
 - Tox Functions reveal association with hepatomegaly and steatosis
 - Perturbation of xenobiotic metabolism genes
 - Agrees with hepatomegaly and steatosis
 - Potential for reduced detoxification
 - Potential for cholestasis – agrees with observed bile duct hyperplasia in liver for these animals

Acknowledgement
The authors would like to acknowledge that all datasets are publicly available and may be obtained from the references cited here in this poster.

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Decreased Expression of Critical P450 Genes for Carmustine

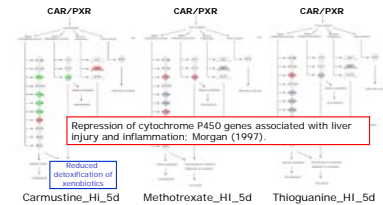


Figure 8: Shown is the impact of high dose 5 day treatments on the CAR/PXR pathway for carmustine relative to methotrexate and thioguanine.

Discriminate Carmustine Specific Effects



Figure 10: Shown is the highest impacted network for the carmustine HI 5d treatment. Overlaying the gene expression charts with expression values for the different drug-dose-time combinations and relevant Toxicity Functions, helps with the understanding and interpretation of the mechanism of toxicity and carmustine's specific effects.