Comparison of Hepatic Gene Expression Profiles from Mice Exposed to Three Toxicologically Different Conazoles

W. Ward, D. Delker, S. Hester, S.-F. Thai, J. Allen, C. Jones, D. Wolf, and S. Nesnow

Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711

United States Environmental Protection Agency

Introduction

Conazoles comprise a class of fungicides used in agriculture and pharmaceutical products. The fungicidal properties of conazoles are due to their inhibition of ergosterol biosynthesis. Certain conazoles are tumorigenic in rodents; both propiconazole and triadimefon are hepatotoxic and hepatotumorigenic in mice, while myclobutanil is not a mouse liver tumorigen. As a component of large-scale studies aimed at determining the relative toxicities and mode(s) of action for tumorigenic and nontumorigenic conazoles, we employed traditional approaches to evaluate comparative P450 enzyme activities and liver toxicity/pathology effects of propiconazole, triadimefon and myclobutanil.

Male CD-1 mice were treated in the feed for 4, 30 and 90 days with triadimefon (0, 100, 500 or 1800 ppm), propiconazole (0,100, 500 or 2500 ppm) or myclobutanil (0, 100, 500 or 2000 ppm). Alkoxyresorufin O-dealkylation (AROD) assays indicated that all 3 chemicals induced similar patterns of dose-related increases in pentoxyresorufin O-dealkylation (PROD), and to a lesser extent, ethoxyresorufin O-dealkylation (EROD) and/or methoxyresorufin O-dealkylation (MROD). However, some chemical-specific differences in the degree of enzyme induction were observed. Propiconazole high-dose exposures over 30 and 90 days induced 36 to 38-fold higher levels of PROD as compared with controls. Myclobutanil and triadimefon at high-dose exposures induced 10 to 15-fold higher levels of PROD as compared to controls at 4, 30 and 90 days.

All high-dose chemical treatments increased liver weights. Liver histopathological changes following exposures to the 3 conazoles revealed similar patterns of dose-dependent increases in cell hypertrophy; however, this effect was most pronounced for propiconazole and triadimefon. High-dose exposures to propiconazole and myclobutanil, but not triadimefon, were associated with early (4 days) increases in cell proliferation. Overall, the tumorigenic and nontumorigenic conazoles induced similar effects on mouse liver P450 enzyme levels and toxicity/pathology..

Research Goal

These results do not provide support for the hypothesis that differential induction of xenobiotic metabolizing P450 enzyme activity is a key determinant of conazole liver tumorigenicity. Toxicogenomic approaches for exploring key gene pathways might identify alternative hypothesis for hepato-tumorigenesis in mice. RNA samples from triplicate medium and high dose mouse livers for each conazole at 4, 30(low dose also) and 90days were analyzed with Affymetrix Mouse 430-2 chips for global gene expression. Preliminary results from this analysis are presented on this poster.

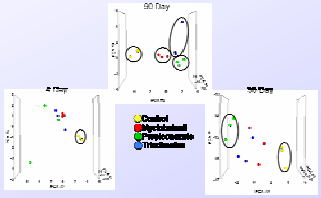
Gene Expression Analytical Techniques

Gene expression measures for each microarray were obtained from Robust Multiarray Analysis of Affymetrix mouse 430-2 Cel files. All 4 day analysis was done using Excel for data tables and GeneSpring for Venn Diagrams and significantly altered genes. Fold change was calculated as the ratio of the mean of three experimental expression measures to the mean of three control expression measures. Functional groupings of genes and their hypergeometric statistics were determined with Ingenuity Systems and Database for Annotation, Visualization and Integrated Discovery (DAVID). Principal Component Analysis was done with Cluster and SigmaPlot. **All analysis presented is for the high dose unless otherwise indicated. The high dose is carcinogenic for triadimefon and propiconazole but not for myclobutanil.**

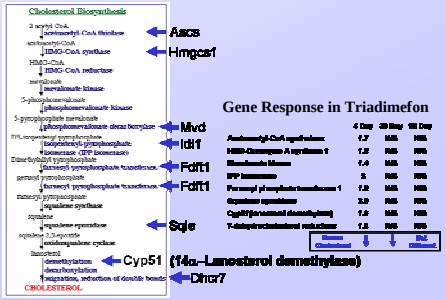
Significantly Altered Genes

	Myclobutanil	Propiconazole	Triadimefon
4 Day			
Mid-Dose	857	25	27
High-Dose	620	1606	516
30 Day			
Low-Dose	10	40	284
Mid-Dose	117	108	2018
High-Dose	353	2622	1340
90 Day			
Mid-Dose	46	1	943
High-Dose	924	1641	2186

Principal Component Analysis



Cholesterol Biosynthesis

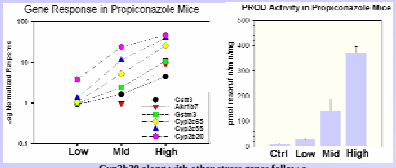


Gene Response in Triadimefon

	4 Day	30 Day	90 Day
Acetoacetyl-CoA synthetase	2.7	NRS	NRS
HMG-Coenzyme A synthase 1	1.8	NRS	NRS
Mevalonate kinase	1.4	NRS	NRS
IPP isomerase	3	NRS	NRS
Farnesyl diphosphate farnesyltransferase 1	1.8	NRS	NRS
Squalene epoxidase	3.9	NRS	NRS
Cytochrome P450, family 51	1.8	NRS	NRS
7-dehydrocholesterol reductase	1.8	NRS	NRS

Lower levels of serum cholesterol correspond with transcriptional upregulation of cholesterol biosynthesis genes at 4 days possibly thru a feedback mechanism. Other regulatory mechanisms override this correspondence at 30 days.

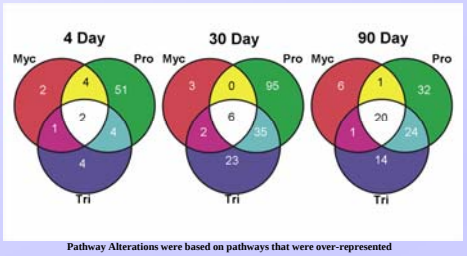
Stress Response



Cyp2b20 along with other stress genes follow a dose response in correspondence with PROD activity. Cyp2b20 is associated with PROD activity.

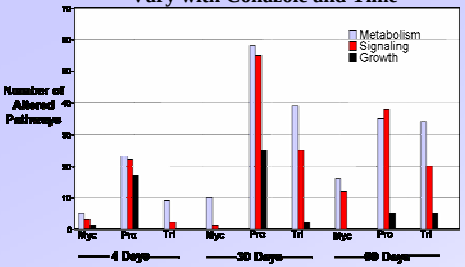
Conazole Pathway Comparisons

Pathway Alterations



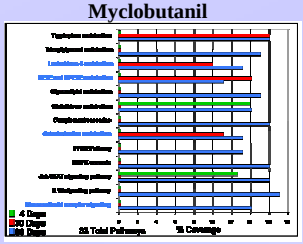
Pathway Alterations were based on pathways that were over-represented with Fisher Exact Test P-value < 0.05 and that had more than 4 genes that were significantly changed.

Metabolism, Signaling, and Growth Pathways Vary with Conazole and Time

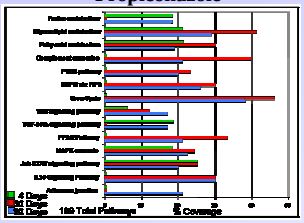


Conazole Significantly Altered Pathways

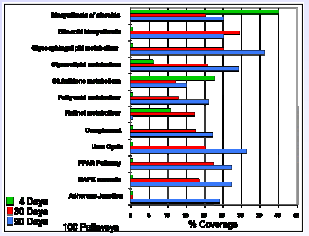
Myclobutanil



Propiconazole



Triadimefon



Tumorigenic Conazole Pathways

Triadimefon Unique Pathways

- Phosphatidylinositol Signaling
- Insulin Growth Factor signaling
- p53-dependent apoptosis
- 14-3-3 proteins in cell cycle regulation
- Biosynthesis of steroids

Propiconazole Unique Pathways

- BAD phosphorylation
- PTEN pathway
- AKT signaling
- Insulin receptor signaling
- BRCA1 as transcription regulator
- Estrogen receptor signaling
- PDGF signaling via STATs and NF-kB
- Regulation of lipid metabolism via LXR
- Role of CD28 in cytoskeleton reorganization
- TGF-b Signaling
- WNT Signaling

Triadimefon and Propiconazole Pathways

- Apoptosis
- Cell Cycle: G2/M Checkpoint
- EGFR signaling
- Regulation of Lipid Metabolism via PPAR,RXR and VDR
- Urea Cycle

Conclusions

- Triadimefon lowers serum cholesterol and upregulates cholesterol biosynthetic genes at 4 days of treatment.
- All conazoles upregulate stress response genes in a dose dependent manner.
- Each conazole has a distinct gene expression pattern that varies significantly over time.
- Each conazole alters a distinct set of pathways that vary over time.
- The mouse liver tumorigens alter significant pathways involved in metabolism, growth and cell cycle regulation.