Application of Toxico-omics to Improving Risk Assessments-Mode of Action of Conazoles

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LTG 1 Poster 01

Science Questions

Can toxico-omics and related approaches be applied to deduce key events and modes of actions of conazoles for cancer and toxicity?

Can "omics" results be incorporated into cumulative risk assessments of these agents?

Can "omic" results be used as a bridge to infer common biologic processes across species (mouse to human) in human relevancy assessments?

Research Goals

To integrate emerging "omics" technologies with traditional laboratory investigations to investigate the modes of action of conazoles.

To employ activity-inactivity pairs of conazoles to allow sequestration of the "omic" results toward known phenotypic endpoints. This may provide a rapid method to identify genes, pathways, and networks that could be responsible for the observed toxic effects.

To provide understanding on the relative contributions of conazoles' abilities to induce and inhibit mammalian CYPs. This knowledge will assist in part, to explain their varied toxic actions.

Studies have been conducted on MOAs of mouse liver tumorigenesis, rat thyroid tumorigenesis, and reproductive toxicity in rats. Here, we will present the MOA of conazole induced mouse liver tumorigenesis.

Toxicological studies of three conazoles indicated that hepatic CAR activation, Cyp2b induction, hypertrophy, and cell proliferation could not fully explain their tumorigenic activities. Genomics data have been used to *discover* key events in the hepatotumorigenic MOA of conazoles. Genomics data have also been integrated with toxicological observations to *augment* the MOA determinations.

A series of key events in the carcinogenic MOA of conazoles have been identified. They are: nuclear receptor activation, Cyp induction, oxidative stress, cholesterol inhibition, effects on retinoic acid and mevalonic acid levels, cell proliferation, apoptosis, and the induction of *in vivo* mutagenicity. These key events have been synthesized into a MOA that helps to explain the toxicological effects of conazoles.

Findings and Conclusions

Two key events have been observed that distinguish mouse hepatotumorigenic conazoles from non tumorigenic conazoles: *in vivo* mutagenicity and increases in hepatic mevalonic acid & anabolite levels.

***In vivo* mutagenicity:** Both propiconazole and triadimefon induced ~ 2-fold increases in mutant frequency while myclobutanil had no significant effect.

Increases hepatic mevalonic acid & anabolite levels: Both propiconazole and triadimefon increased these levels while myclobutanil did not.

"Omics" data can be used to infer key events in the MOA; to perform human relevancy determinations; to screen new chemicals for common key events; and to assist in cumulative risk assessments of groups of conazoles.

Impact and Outcomes

•The default MOAs for hepatotumorigenesis in the mouse and thyroid tumorigenesis in the rat by these agents were disproved, and new modes of action were proposed.

•OPP/NHEERL is re-evaluating the MOA for hepatotumorigenesis in the mouse for selected conazoles and will use this new mechanistic information in the application of the Framework for MOA analysis.

•ORD/NHEERL is scheduled to present the MOA of these conazoles and to demonstrate how "omics" can be incorporated into cancer risk assessment activities to OPP's SAP in 2010.

Future Directions

1. Apply "omic" technologies to the human relevancy framework to complete the MOA analysis of conazoles. Use "omic" data obtained from human hepatocytes.

2. Extend "omic" technologies to perform cumulative risk assessment of the class of conazoles. Evaluate additional conazoles for their MOA. Find common biomarkers/ key events.

3. Apply this approach to other classes of environmental agents such as amide herbicides and oxazole herbicides.

Researchers Involved

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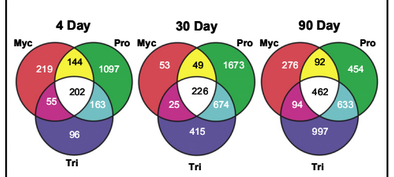
Approach to conazole MOA research: Criteria for chemical selection: activity-inactivity pairs

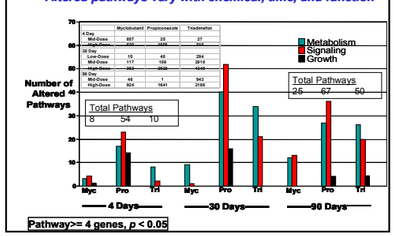
Triadimefon (Bayleton)
mouse: hepatocellular adenoma
Propiconazole (Orbit)
mouse: hepatocellular adenoma/carcinoma
Myclobutanil (Eagle)
mouse: no liver tumors

While toxicological studies in the mouse revealed that all three conazoles had similar effects on liver hypertrophy, AROD activities, cell proliferation, and serum cholesterol, genomic analyses suggested that there were major differences in their gene expression profiles. These differences were used to the identification of the important key events leading to their hepatotumorigenic activities.

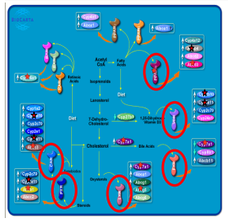
Significantly altered genes with high dose conazole treatments



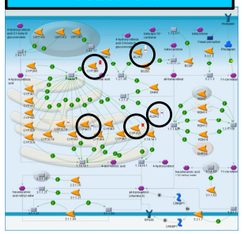
Altered pathways vary with chemical, time, and function



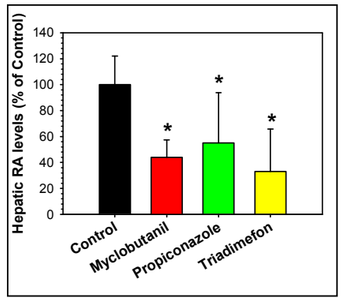
Activate CAR, PXR



Increase atRA metabolism/ decrease atRA levels



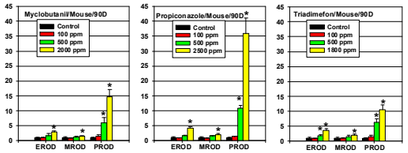
Conazoles Decrease Hepatic RA Levels



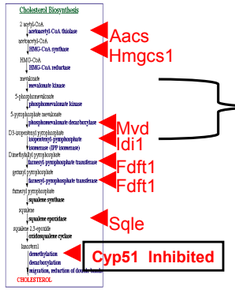
Key Events Informed by Genomics

Induce high activities of Cyp2b, Cyp3a

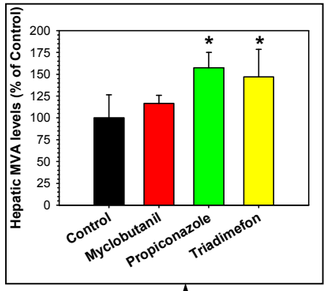
Conazoles induce alkoxyresorufin dealkylase (AROD) activities in mouse liver microsomes



Inhibit Cyp51, reduce serum cholesterol, increase expression of cholesterol biosynthesis genes

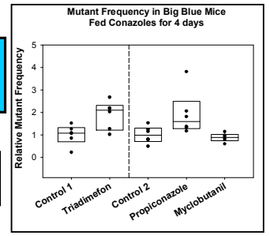


Increase hepatic mevalonic acid & anabolite levels



Key Events That Differentiate Tumorigenic and Non-Tumorigenic Conazoles

Indirect mutation in vivo



Proposed MOA for Propiconazole Mouse Liver Tumorigenesis

Propiconazole parent

Activate CAR, PXR

Induce high activities of Cyp2b, Cyp3a

Hypertrophy

Indirect mutation in vivo

Inhibit Cyp51, reduce cholesterol

Increase atRA metabolism/ decrease atRA levels

Increase cell proliferation/alter cell signaling

Increase mevalonic acid

CYP generated OH radical/oxidative stress

Increase in preneoplastic foci?

Liver Tumors

Time

Legend
Key event measured
Key event implied

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Use of Mechanistic Data in Risk Assessment