Metabolism-mediated Toxicity

ORGAN Tox Panel

For the prediction of liver metabolism cytotoxicity

Any relatively non-toxic xenobiotic may be metabolized by the liver into toxic products (metabolic activation). These can easily reach organs distant from the site of metabolism (e.g. peripheral neurons, brain), where they then cause toxic effects.

As an example, many species of Aspergillus, a fungus, produce mycotoxins such as aflatoxins. Aflatoxins are toxic and among the most carcinogenic substances known. Upon arrival in the liver, aflatoxin B1 is converted by CYP3A to a highly reactive hepatotoxic epoxide intermediate.

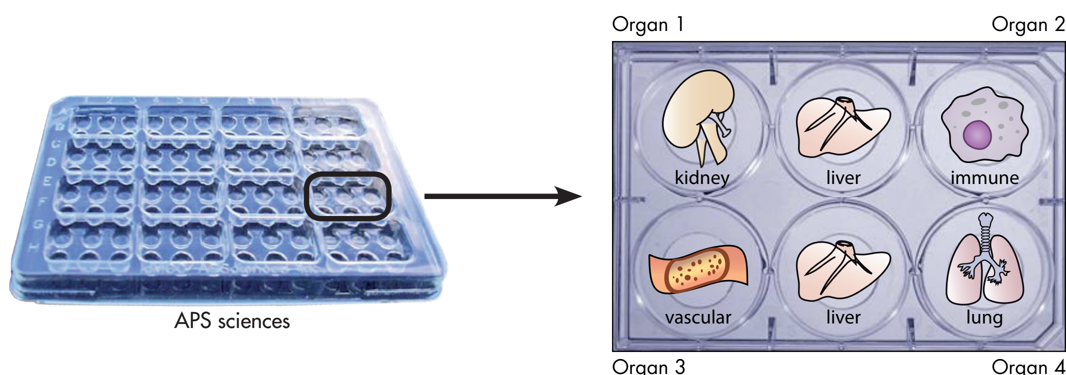
The opposite can also be true: a metabolite can be less toxic than the parent compound. This is the case of terfenadine and its CYP3A metabolite, fexofenadine. Terfenadine, marketed as seldane, was a leading antihistamine until cardiac toxicity was discovered among patients.

Assay systems need to be able to assess whether the liver might metabolize the test compound to either a more or less toxic moiety.

Experimental approach

Integrated discrete Multiple Organ Culture (IdMOC), developed by Dr. Albert Li, Ph. D. of Advanced Pharmaceutical Sciences, Inc., is a technology allowing the culture of cells from multiple organs in the same culture dish. The IdMOC plate consists of multiple, inner wells within a larger interconnecting chamber. Multiple cell types are individually cultured in the inner well and the chamber is filled with a single, universal medium, allowing well-to-well communication. The overlying medium can be analyzed for test material metabolism, and individual cell types can be evaluated for possible organ-specific cytotoxicity[1].

Using IdMOC plates, we cocultured primary cells representing different tissues (kidney, endothelium, immune, lung) in the absence or presence of human hepatocytes to assess cytotoxicity of drug and related metabolites, respectively. To validate the technology, we evaluated the cytotoxicity of a compound that requires metabolic activation (aflatoxin B1), and an agent whose metabolite is known to be less toxic than the parent compound (terfenadine). For this purpose, compounds were incubated for 48 hours in IdMOC plates in the presence of a metabolically-competent cell type (human hepatocytes) or a metabolically non-competent cell type (chinese hamster ovary (CHO) cells). Cytotoxicity was determined in each cell type by measuring ATP production as an indicator of cellular respiration.



ORGANS	CELLS[2]	ASSAYS
RENAL	Proximal tubular cells (RPTEC)	
VASCULAR	Endothelial cells (HUVEC)	
LIVER	Hepatocytes	Metabolism: ATP
IMMUNE	Macrophages	
LUNG	Epithelial cells (NHBE)	

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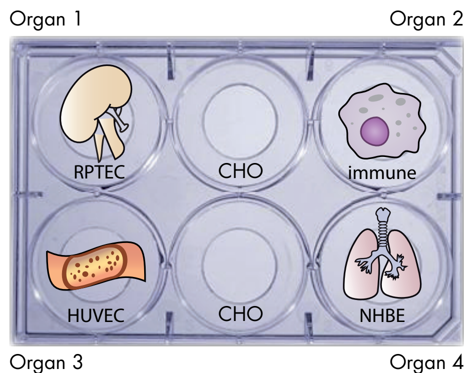
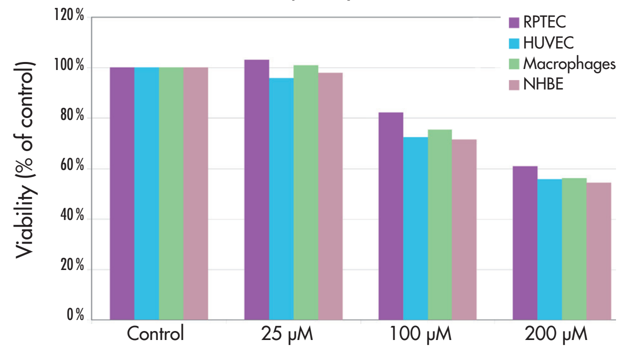
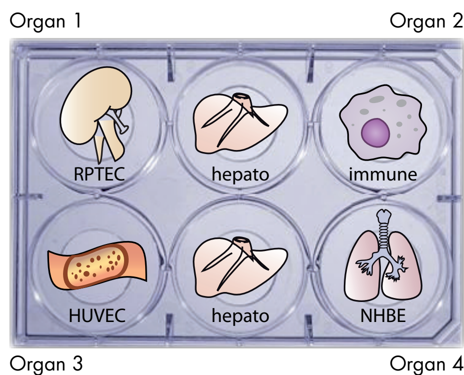
Results

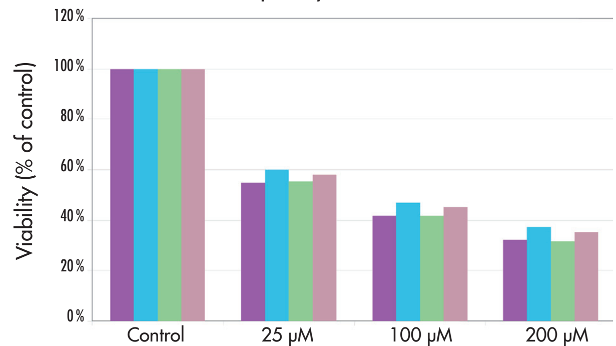


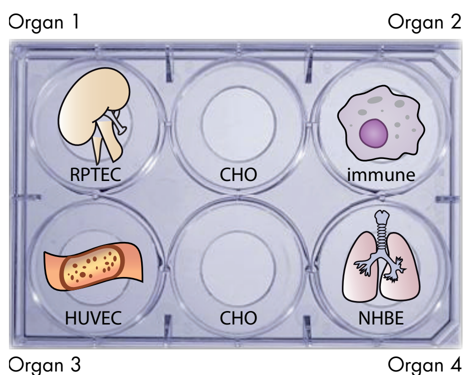
Aflatoxin B1 without hepatocytes



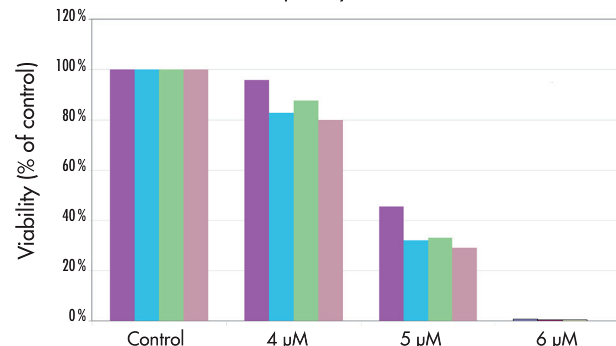


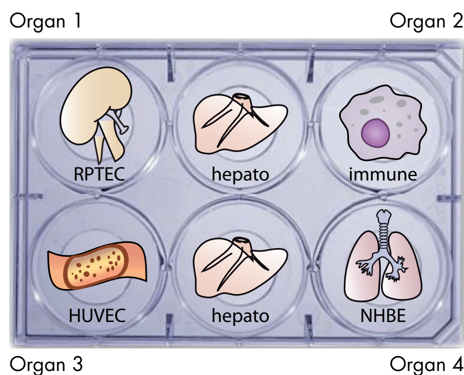
Aflatoxin B1 with hepatocytes



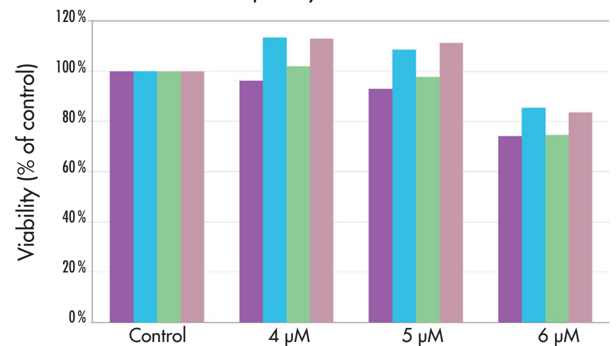


Terfenadine without hepatocytes





Terfenadine with hepatocytes



An IdMOC experiment with cocultures of human hepatocytes and CHO cells confirmed that aflatoxin B1 requires metabolic activation to be cytotoxic. Indeed, aflatoxin B1 was more cytotoxic on the different cell types when human hepatocytes were present. Conversely, terfenadine is metabolically inactived since the parental compound was made less cytotoxic when human hepatocytes were present.

Cerep proposes the use of IdMOC technology to assess metabolism-mediated cytotoxicity on primary cells representing multiple organs. In order to assess the cytotoxicity of drugs and metabolites, experiments will be conducted in the absence and presence of human hepatocytes, respectively.

1. ATLA 37, 377–385, 2009

2. Primary cells, gift from Lonza