Metabolically, man or mouse?

By Tracey Baas, Senior Editor

Massachusetts researchers have created mice with human ectopic artificial livers that were used to evaluate human drug metabolism, drug-drug interactions and drug-induced liver injury in a proof-of-principle study.¹ Unlike animal models that are currently under development for monitoring liver toxicity, the new mice are healthy and immunocompetent, which might give a better picture of outcomes in humans.

The artificial livers consist of a porous polyethylene glycol (PEG) scaffold encapsulating three cell types: human hepatocytes to carry out the liver's metabolic functions and mouse fibroblasts and human liver endothelial cells to provide biological and chemical signals necessary for hepatocyte function (see **Figure 1, "Human ectopic artificial liver mouse"**).

In addition to providing support, the scaffold helps prevent the mouse immune system from attacking the transplanted cells, allowing the livers to be implanted for up to eight days in immunocompetent mice or indefinitely in immunocompromised animals.

Gene expression assays confirmed that the livers expressed cytochrome P450 3A4 (CYP3A4), CYP1A2, CYP2D6, CYP2E1 and CYP2C, which together metabolize more than 90% of known drugs.

In mice with artificial liver implants that received the anticoagulant coumarin or the antihypertensive debrisoquine, metabolites of those drugs that are observed in humans but not in mice turned up in serum and urine. That finding indicated the activity of human liver CYP2A6 and CYP2D6 enzymes and suggested the mice could be used to study human drug metabolism.

The researchers also used the engineered mice to recapitulate the effects of a known drug-drug interaction between the antibiotic rifampin, a cytochrome P450 inducer, and the analgesic acetaminophen. Animals receiving the combination had greater hepatocellular injury, indicative of higher levels of the hepatotoxic metabolite *N*-acetyl-*p*-benzoquinone, than mice given either compound alone.

The findings were published in the *Proceedings of the National Academy of Sciences*.

The team was led by Sangeeta Bhatia, professor of health sciences and technology, and electrical engineering and computer science at the **Massachusetts Institute of Technology** and chair of **Hepregen**

Corp.'s scientific advisory board. The paper also included researchers from MIT, **Harvard University**, the **Broad Institute of MIT and Harvard**, the **Dana-Farber Cancer Institute** and **Brigham and Women's Hospital**.

"The previous approach of injecting human hepatocytes into immunodeficient mice with liver injury to repopulate the liver provides a liver with a mixed metabolism" of both human and mouse, said Alice Chen, first author on the paper and a graduate student in Bhatia's laboratory. "This method of humanization is highly unpredictable and variable and produces mice that are not very healthy. Human ectopic artificial livers can be implanted into normal, healthy mice and provide consistent results."

Model citizens

Next steps include using the mice to validate known metabolic characteristics and drug-drug interactions in larger panels of marketed drugs, as well as adapting the model for drug development of human liver diseases, including HCV and malaria infection.

Nico Scheer, head of ADMET R&D at **Taconic Farms Inc.**, thinks "the human ectopic artificial liver mice might indeed be useful for studying human metabolism and drug-drug interactions in late-stage drug development ADMET studies."

He did say the approach also has certain limitations. For example, the presence of mouse hepatocytes might confound metabolic

interpretation.

Scheer and collaborators recently produced a mouse model using a targeted insertion strategy to humanize four genes involved in drug metabolism: pregnane X receptor (PXR), constitutive androstane receptor (NR1I3; CAR), CYP3A4 and CYP3A7. The group used the model to predict human drug-drug interactions² and now is working toward more complex mouse lines with additional humanization. The group believes the model may avoid the use of nonhuman primates for some drug-drug interaction studies.

Salman Khetani, director of research at Hepregen, said the mice with humanized ectopic artificial livers will have to be more thoroughly evaluated to see if they are better able to predict clinical outcomes than existing model systems.

The key question, he said, is whether the mice "can be used in a scalable business model while reducing clinical trial attrition rates and liabilities. They will be better positioned to answer that question after using their mice to characterize larger panels of marketed drugs to validate known metabolic characteristics."

Hepregen is playing further upstream in the drug development process than mouse models of drug-drug interactions. The company has commercialized a micro liver chip platform, HepatoPac, through a service offering high throughput screening of large panels of drugs in stable human hepatocytes.

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—Nico Scheer, Taconic Farms Inc.

Figure 1. Human ectopic artificial liver mouse. Primary human hepatocytes are cocultivated with stabilizing stromal mouse fibroblasts on collagen-coated plates for 7–10 days. They are then encapsulated with human liver endothelial cells in polyethylene glycol–diacrylate (PEG-DA) scaffolds derivatized with adhesion Arg-Gly-Asp-Ser (RGDS) peptides. The human ectopic artificial livers (HEALs) are about 20 mm in diameter and 250 μm thick, with about 0.5×10^6 human hepatocytes. They are surgically placed intraperitoneally into laboratory mice, in which the implants become engrafted and vascularized. The HEAL mice provide models to study human metabolites, drug exposure via different routes, drug-drug interactions and drug toxicity. (Figure based on Figure 1 in ref. 1.)

“We have used HepatoPac to predict 75%–80% of known clinical toxicities in a panel of 200 different drug candidates and have identified up to 82% of clinical drug metabolites in a panel of 32 compounds with **Pfizer Inc.**^{3,4} The platform is capable of evaluating human drug metabolism, drug-drug interactions and drug-induced hepatic injury,” said Khetani. “*In vitro* and *in vivo* models address different parts of the drug development pipeline—they speak to a different audience.”

MIT has filed for a patent covering the mice. The model is available for licensing.

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