Mice with 'humanized' livers improve early drug testing

October 31 2012

Stanford University School of Medicine scientists have used bioengineered mice with livers composed largely of human cells to characterize a drug about to enter early-stage clinical development for combating hepatitis C.

Tests using the new mouse model accurately predicted significant aspects of the drug's behavior in humans—including its interaction with another drug and the profile of its major breakdown products in the body (called metabolites)—far more accurately than would have been achieved using current methods.

The study will be published online Oct. 31 in the Journal of Pharmacology and Experimental Therapeutics. Its findings hold potentially huge implications for drug development in general, because key aspects of the tested drug's activity and properties would likely have gone unnoticed using the kind of mouse study that is the current standard for preclinical tests of candidate drugs. Importantly, the results strongly hint that the drug, clemizole, could be both safe and an effective drug-cocktail component in humans infected with HCV, the virus that causes hepatitis C.

"This gives us a new tool for improving the testing of drugs before they are given to people in clinical trials," said the study's senior author, Gary Peltz, MD, PhD, professor of anesthesiology, pain and perioperative medicine.

All too often, drugs showing tremendous promise in preclinical animal assessments fail in human trials because of unforeseen safety problems, said Peltz. "It's often not the drug itself, but one of its metabolites, that is responsible for a drug-induced toxicity."

Unexpected interactions between drugs pose another big problem for drug development. A drug may prolong or attenuate another medication's activity by, for example, affecting how the second drug is metabolized. With more than 30 percent of all people over age 57 taking five or more prescription drugs at any given time, that's no trivial matter.

The drug tested in the study, clemizole, was widely prescribed in the 1950s and 1960s as an antihistamine, but it is no longer used because more effective antihistamines now exist, said Jeffrey Glenn, MD, PhD, associate professor of gastroenterology and hepatology, and of microbiology and immunology. "Moreover, the drug tends to accumulate in the liver, which is not ideal for a general-purpose antihistamine but could be very attractive for a virus like HCV that only infects the liver," he said.

Glenn, who is a hepatitis C expert and a co-author of the new study, recently led a team that discovered clemizole impedes replication of HCV. More than 150 million people are infected with HCV, the leading cause of liver transplant operations in the United States and primary cause of liver cancer. Current HCV treatments are highly expensive and, frequently, harsh.

Clemizole is both cheap and safe. But because it was approved before the advent of some testing requirements now routinely in place for new drugs, little is known about how the compound is metabolized or how it interacts with other drugs in the human body, Glenn said.

These days, before any drug can go into people it must first be rigorously

tested in animals, such as rodents, to determine tolerability or adverse effects and whether it interacts with other drugs patients are likely to be taking. But mice metabolize things differently from humans, largely because our livers are different.

The liver is the body's chemistry set. It operates like a set of carefully placed workstations in an assembly line, in which batteries of enzymes (protein machines that do most of the body's work) manufacture substances vital to our survival as well as metabolize ingested substances, including drugs.

Mouse and human livers have different drug-metabolizing enzymes. So the two species will produce different metabolites or different amounts of the same metabolites from the same drug. Attempts to get around this have included bioengineering so-called chimeric mice that have "humanized" livers, in which mouse liver tissue has been at least partly replaced by human cells. These efforts have involved introducing toxins or genetic defects to kill off the intrinsic mouse liver cells to make room for their replacement by human ones. But the organ's ongoing malfunction impaired human-cell growth or made "readouts" from drug testing suspect.

So Peltz and collaborators at the Central Institute for Experimental Animals in Japan built a better mousetrap. In 2011, they produced a genetically engineered mouse in which the liver could be humanized without inducing ongoing liver toxicity. The researchers administered a short-acting, non-toxic dose of a drug to mice that had been bioengineered so that the drug would activate a cell-killing mechanism only within their liver cells. Once this drug was cleared, the implanted human liver cells could develop normally in their new environment, contributing to a reconstituted liver that largely recapitulated the architecture and chemistry of a functioning human liver. The human cells produced human metabolites; the mouse cells continued to produce

mouse metabolites.

The chimeric mice used in the new study varied in the extent to which their livers were composed of human cells. Their overall metabolic profiles could be likened to two images projected in juxtaposition on a screen, with the difference between the two images corresponding to the extent that a mouse's liver had been humanized. To determine the extent of liver humanization for each mouse, Peltz and his associates measured blood levels of the human version of albumin, a circulating protein produced in the liver. A mathematical algorithm the researchers developed allowed them to accurately determine which metabolites, and how much of each, could be attributed to mouse and human liver cells, respectively.

Next, Manhong Wu, PhD, a research associate in the Peltz lab and a study co-author, examined the metabolism of clemizole in both humans and several ordinary mouse strains. Clemizole's metabolic pattern was the same in all of the tested strains, but was quite different from that observed in the blood of 10 human subjects. More than half of the total amount of clemizole plus its metabolites in human blood consisted of a single metabolite, known as M1. In ordinary mice, M1 is a trace product.

However, postdoctoral scholar Yajing Hu, PhD, who shares first authorship with visiting professor Toshiko Nishimura, MD, PhD, found that the chimeric mice did produce M1, roughly in proportion to the extent to which their own liver cells had been replaced by human ones. Further studies showed that M1 itself has antiviral activity that can contribute to clemizole's overall potency in humans—a fact that would have been ignored on the basis of testing clemizole in ordinary mice.

Then Peltz and his colleagues tested the chimeric mice's capacity to predict potential interactions between clemizole and other drugs. They picked a drug called ritonavir, which is known to interfere with a

metabolic enzyme that is crucial to the breakdown of many drugs in humans. Chimeric mice were first treated with clemizole alone, and later given a combination of clemizole and ritonavir. Afterward, the scientists measured levels of clemizole and its metabolites in the mice's blood. Co-administering ritonavir caused clemizole's blood level to increase and to remain elevated for longer than was the case with clemizole alone.

To see if this held true in humans, Peltz's group initiated a small pilot study with three HCV-positive individuals. As occurred in the chimeric mice, co-administration of ritonavir caused a substantial increase in the blood levels of clemizole in two subjects and a smaller increase in the third subject.

In lab-dish tests assessing clinical potential, a combination of M1 (clemizole's primary metabolite) and boceprevir, a recently approved anti-HCV drug, proved to have far more anti-viral activity than did either compound alone—a synergy that Glenn called "dramatic."

All of the findings validate the utility of the new mouse model as well as clemizole's clinical potential, said Peltz. Clemizole has a half-life of only about 15 minutes in mice, so on the basis of ordinary mouse studies it might well have been discarded, he said. "You can't commercialize a drug you have to take 10 or 20 times a day." Further, Peltz said, "if clemizole's major human metabolite, M1, did cause toxicity in humans, ordinary mouse tests wouldn't have caught it. Conversely, if a metabolite produced in mice but not in humans had a toxic effect, studies performed on ordinary mice would have sent a false alarm."

Provided by Stanford University Medical Center