'Clinical trials in a dish' may be more reliable than standard way of measuring drug effects on heart, researchers say

March 22 2013, by Krista Conger

(Medical Xpress)—Last week, the common antibiotic Zithromax received a new warning label from the U.S. Food and Drug Administration indicating it could cause dangerous arrhythmias in people with pre-existing heart conditions. Today, researchers at the Stanford University School of Medicine describe a "clinical trial in a dish" using patient-specific induced pluripotent stem, or iPS, cells to predict whether a drug will dangerously affect the heart's function. The technique may be more accurate than the current in vitro drug-safety screening assays used by pharmaceutical companies, say the researchers, and may better protect patients from deadly side effects of common medications.

The technique allows scientists for the first time to test drugs directly on cells with mutations that cause hereditary cardiac diseases, rather than on the genetically modified human embryonic kidney cells or the Chinese hamster ovarian cells currently being used to detect cardiac toxicity.

The use of patient-specific iPS cells may help drug designers winnow heart-safe medications from those like the blockbuster anti-inflammatory drug Vioxx, which was withdrawn from the market because of unanticipated adverse cardiovascular events. It may also allow clinicians to identify sub-groups of patients, such as those with certain types of cardiac conditions, who should not be given certain drugs.

"Right now, the first time any drug sees a human heart cell is in a phase-1 clinical trial," said Andrew Lee, a Stanford medical student and one of three lead authors of the study. "If adverse effects are seen, it can result in patient deaths, as in the case of the anti-inflammatory drug Vioxx or with cisapride, a drug previously used to treat digestive problems in people with diabetes. Right now, there are really no systematic studies to identify those people who are at risk." Lee works in the laboratory of Joseph Wu, MD, PhD, who co-directs the Stanford Cardiovascular Institute, where the research was conducted.

The researchers anticipate that the technique, if adopted, could save millions of dollars and thousands of lives by streamlining the drug-testing process and increasing its sensitivity.

It may also lead, simply, to better medicine, said Wu, who is also an associate professor of cardiovascular medicine and of radiology. "Our hope is that, instead of a physician using a patient as a guinea pig, trying one medication after another until something is found to be effective, this method will one day lead to personalized drug screening to find out exactly which medication is the best for you."

Wu is the senior author of the study, which was published today in *Circulation*. In addition to Lee, lead authors of the study are postdoctoral scholars Ping Liang, PhD, and Feng Lan, PhD.

Currently, all drugs under consideration by the FDA for use in humans must be shown to be nontoxic to the heart—regardless of the condition they are meant to treat. But because of the difficulty of collecting and growing human heart tissue in the laboratory, drug developers for a long time have tested drugs on kidney cell lines or hamster ovary cells. (Tissue from animal hearts is not a good option because they beat at different rates and have different electrophysiological properties than human heart tissue.)

Although neither of these cells are heart cells, they are genetically engineered to express a single human cardiac ion channel called hERG, upon which researchers can test the effect of various potential drugs. The assumption has been that these single-channeled, non-heart cells can serve as handy doppelgangers for true human heart muscle—at least for the purpose of determining drug toxicity.

However, hERG is just one of many ion channels in the cells of human-heart muscle. These channels work together in complex ways to control and synchronize the beating of the cells in response to waves of charged molecules called ions. And it's clear from examples like Vioxx, cisapride and others that these "heart" cells are a poor substitute for the real thing when it comes to predicting how patients will respond.

The human iPS cell technology was invented in 2007 by Shinya Yamanaka, who received the 2012 Nobel Prize in Medicine or Physiology for his discovery. In the present study by Stanford investigators, the human heart cells were created by taking painless, noninvasive skin samples from people with and without inherited cardiac diseases, such as familial hypertrophic cardiomyopathy, familial dilated cardiomyopathy and hereditary long QT syndrome. The skin cells were first coaxed to become iPS cells and then differentiated into beating heart cells so that the researchers could easily grow and study them. (The researchers used a similar technique to create heart cells from a human embryonic stem cell line to use as positive control.)

The researchers created the iPS-cell-derived heart cells from three members of each family who had inherited the disease, and one member per family who had not. They confirmed that those cells from the affected patients displayed characteristics such as abnormal cell size, organization, gene expression and rhythms of contraction that mimicked the clinical symptoms seen in patients with that condition. In contrast, cells from unaffected family members resembled heart cells created

from normal human embryonic stem cell lines without the disease-causing mutations. The researchers also showed that the iPS-cell-derived heart cells expressed the same genes for multiple cardiac ion channels as surgically isolated adult human heart tissue.

Next they tested how the cells, growing in a laboratory tissue culture dish, responded to drugs. One, verapamil (a drug marketed under several names that is used to treat a variety of conditions, including hypertension and cardiac arrhythmias), is known to block the hERG channel, but is not generally cardiotoxic. The other, alfuzosin (a drug used to treat benign prostatic hyperplasia, marketed as Uroxatral), appeared safe in traditional cardiac toxicity screens but can be dangerous to patients with long QT syndrome. A third, cisapride (a drug used to treat reflux and constipation, marketed as Propulsid), was withdrawn from the market in 2000 because of cardiotoxicity.

When the researchers compared the responses of the iPS cells from patients with pre-existing cardiac conditions with those of heart cells from human embryonic stem cells and hERG-expressing human embryonic kidney cells, they found a significant difference. Although the hERG-expressing cells indicated that verapamil was highly likely to be cardiotoxic, the embryonic stem cells and iPS cells showed that the drug was unlikely to cause dangerous arrhythmias at the amounts normally prescribed.

The findings for alfuzosin were also interesting. Although the medication did not affect the hERG channel in the kidney cells, it did significantly affect the rhythm of the heart cells derived from both human embryonic stem cells and from patients with cardiac conditions. Finally, tests of cisapride indicated that the medication affected each of the patient-specific cell lines in varying ways as the drug concentration was increased, suggesting there may be a range of doses for which the drug would be safe for patients without heart disease.

"In these instances, our stem-cell-based testing platform is more sensitive and accurate than the current industry standard, which can lead to false negatives and false positives," said Liang, the co-lead author of the study. "As a result, it may be a better way to test medications in pre-clinical trials."

"It's clear that individual patients will respond uniquely to specific drugs," Lee said. "If you have a hereditary disease or a problem with your ion channels, you're going to respond differently than members of the general population. Even companies relying on genetically normal human embryonic-stem-cell-derived cardiac cells won't be able to see all these effects. But our 'clinical trial in a dish' with patient-specific iPS cells allows us to model this personalized response and identify high-risk groups who should not receive the drug."

The technique could also allow researchers to test combinations of drugs on the cells to assess safety for patients on multiple, possibly interacting, medications.

"This study shows that the use of patient-specific stem cells to detect cardiotoxic properties of pharmaceutical compounds may be more accurate than the current drug-safety assays mandated by the FDA," Wu said. "We are also able to demonstrate disease-specific responses to cardiotoxic drugs. We believe that, in the future, this may become a standard way to test drug safety and efficacy."

Provided by Stanford University Medical Center