Clinical Chemistry

Sandwich Assay for Tacrolimus Using 2 Antitacrolimus Antibodies

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Guest:
Dr. Tie Q. Wei is in the research and development department of Siemens Healthcare Diagnostics in Delaware.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I'm Bob Barrett. Tacrolimus or FK506 is a macrocyclic lactone that is commonly used along with other immunosuppressant drugs to reduce graft rejection in organ transplantation by suppressing the immune system. Because of its narrow therapeutic window, it is critical to accurately monitor blood concentrations of this drug for optimal efficacy.

In the April 2014 issue of *Clinical Chemistry*, a sandwich assay for tacrolimus using two antitacrolimus antibodies was described by researchers at Siemens Healthcare Diagnostics. We're joined by the lead author of that study, Dr. Tie Q. Wei. And Dr. Wei, tell us about the importance of monitoring drug concentrations in patients' blood.

Dr. Tie Q. Wei: Many drugs have a very narrow therapeutic window; when the dose is too high the drug can be toxic to patients, but if it is too low it won't be effective. High doses of tacrolimus, for example, can result in nephrotoxicity, and renal toxicity of tacrolimus appears to be dose-dependent. Other side effects can include infection, tremor, headache, stomachache, and anemia.

So, with immunosuppressive drugs like tacrolimus, while the circulating levels must be high enough to prevent rejection of transplanted organs, it is very important to keep the dosage low enough to minimize the side effects. For that reason, monitoring drug concentrations in patients' blood is needed.

Bob Barrett: What are the advantages of a sandwich assay for monitoring therapeutic drugs over the traditional competitive assay?

Dr. Tie Q. Wei: The first major advantage is specificity: tacrolimus is metabolized by the liver to a number of metabolites which often cross-react with the antibody that is used to measure it. These metabolites are not supposed to be measured because most of them are not immunosuppressive and are not really as toxic as the parent drug. So when they cross-react with the assay antibody in the competitive assays, and depending on their levels, they may give false positive

results. In the case of our sandwich assay, the drug needs to fit in the binding pockets of both antibodies, rather than just one. This minimizes the assay cross-reactivity with the metabolites and gives more accurate results—and which actually is closer to that of Mass Spec but with a fraction of the cost.

Second, it is more sensitive because two antibodies in the assay reagent can be in excess relative to the analyte, which allows the detection of very low levels of analyte in patients' blood. High sensitivity is very important for immunosuppressant drugs as transplant surgeons are constantly looking for effective cocktails of low dose immunosuppresants. This can prevent transplant rejection while minimizing the side effects of higher doses of individual drugs. So the drug has to be measured at very low levels.

Another point is: when excess amount of antibodies are used in the reagents, assay precision is less affected by reagent delivery variations from test-to-test. This makes the assay more robust compared to traditional competitive assays that are more reagent delivery dependent.

Bob Barrett: Doctor, why are there very few sandwich assays for small hapten drugs?

Dr. Tie Q. Wei: This is because a sandwich assay requires the hapten to be bound by two antibodies. The two antibodies should bind to different parts, or to what we call "different epitopes," on the hapten. But because hapten molecules are usually very small (<1000 daltons), simultaneous binding by two large antibodies, which are usually 150,000 daltons, is almost always prevented by steric hindrance. Once the drug is bound to the first antibody, it usually fits in a surface pocket and doesn't leave much of the drug molecule exposed, for the second binding by the second antibody. So to come up with a sandwich assay for a hapten drug, you need to make sure the binding sites or epitopes for the two antibodies are approachable from two separate directions, which is difficult for small haptens.

Bob Barrett: So with the know difficulties of developing sandwich assays for small haptens, how did you come up with the idea of the sandwich assay for tacrolimus?

Dr. Tie Q. Wei: It was kind of a serendipitous discovery. While characterizing assay antibodies for the competitive format, we found some drug analogs that bind to either 14H04 antibody or 1E2 antibody, but not both. What's more interesting, although kind of expected, is that the two antibodies demonstrated different cross-reactivity profiles

with the drug metabolites, which indicates that the antibodies should have different binding epitopes on the drug molecule. But the question is: do the two binding epitopes overlap? So we still needed to map the binding epitopes to find this out.

We made the assumption that if you lose antibody binding after modification of a part of the molecule, that part of the molecule is a binding site. So with the cross-reactivity data, we mapped the binding epitopes for the two antibodies. We found that they are distinct and not overlapped. So we tested this prediction and observed dose response signals in a sandwich format.

At first, we were excited about the discovery, and later on debated among us whether this was a fluke or true phenomenon. So we designed a series of experiments that included different assay formats with different tag molecules and detection systems, and with the same antibody for both capture and tag. The studies ruled out other possibilities such as non-specific adsorptions and/or hydrophobic interactions etc. In addition, the cross-reactivity profile of the assay is consistent with the profile predicted by sandwich formation. After that, we were confident that we had a true sandwich assay for tacrolimus.

To our knowledge, this is the first report of a sandwich assay for a natural hapten.

Bob Barrett: Finally doctor, can sandwich assays be developed for other hapten analytes?

Dr. Tie Q. Wei: Yes, we think so, as long as the steric hindrance between the bindings by two binding partners can be avoided. The binding partners can be antibodies, binding proteins, receptors, eptimers (such as DNA/RNA molecules), or anything that binds to the drug with enough specificity and affinity, which can be tagged to solid support and/or signal molecules.

We believe if appropriate techniques and strategies are used, sandwich assays can be developed for more hapten analytes. We hope that the demonstration of this tacrolimus sandwich assay will encourage more research activities that will lead to the development of more sensitive and specific immunoassays for the small haptens.

Bob Barrett: Dr. Tie Q. Wei is in the research and development department of Siemens Healthcare Diagnostics in Delaware. He's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett, thanks for listening.