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High-Resolution Mass Spectrometry for Characterizing the Metabolism of Synthetic Cannabinoid THJ-018 and Its 5-Fluoro Analog THJ-2201 after Incubation in Human Hepatocytes

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Guests: Dr. Marilyn Huestis, Senior Investigator and Chief of the Chemistry and Drug Metabolism section at the National Institute on Drug Abuse, and Dr. Xingxing Diao, a postdoctoral fellow at the National Institute on Drug Abuse.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Synthetic cannabinoids are designer drugs that mimic the effects of cannabis. They are usually applied to an herbal base material and can be found under product names like K2 and Spice. These compounds interact with endogenous cannabinoid receptors and elicit effects similar to those of delta-9-tetrahydrocannabinol, the principle psychoactive constituent of cannabis.

Use of some synthetic cannabinoids has been associated with serious toxicity, including acute kidney injury, myocardial infarction, even death. Despite increasing prevalence of these novel psychoactive substances, there are few human metabolism data currently available.

The January 2016 issue of *Clinical Chemistry,* a special issue devoted to mass spectrometry and the clinical laboratory, published a paper from researchers at the National Institute on Drug Abuse, characterizing the metabolism of two synthetic cannabinoids that also suggest appropriate markers for laboratories to identify intake and link observed averse events.

Two of the authors of that papers join us in this podcast; first Dr. Marilyn Huestis. She is Senior Investigator and Chief of the Chemistry and Drug Metabolism section of the lab, and we will start with you, Dr. Huestis.

Can you explain the importance of novel psychoactive substances in the US and the world?

Dr. Marilyn Huestis: Yes, it's a major problem; in fact, I think that the novel psychoactive substances is the new face of drug abuse in the world.

According to the Drug Enforcement Agency, individuals who are synthesizing and marketing these novel psychoactive substances have a much greater profit margin than they do

with smuggling in cocaine or heroin or other drugs of abuse, and they have very little chance of being caught.

The worse that can happen is that Customs or someone intercepts a package that they are shipping, through the internet, primarily. So I really believe that we are in trouble, and not only do we have synthetic cannabinoids, synthetic cathinones, but now we have synthetic benzodiazepines and synthetic opioids, and the stream of these new compounds don't stop.

In fact, the European Monitoring Centre that is following all of these drugs as they show up in Europe, they have found and identified more than 450 novel psychoactive substances since 2008.

So you can imagine, we spent so many years trying to control cocaine, amphetamines, cannabis, opioids, and just a few other drug groups, and now they are literally hundreds, which is why it has made it so important that our laboratory here at the National Institute on Drug Abuse come up with a strategy to address this.

Bob Barrett: Dr. Huestis, how has your laboratory at the National Institute on Drug Abuse contributed to confronting this public health and safety crisis?

Dr. Marilyn Huestis: Well, as you know, our laboratory conducts controlled human administration studies, and we are able, then, to study all aspects of the drug's effects on the human, as well as the pharmacokinetics. But unfortunately, even though I have tried since 2008, we have not been able to run a single controlled administration study on any of these new compounds, and the reason is because we don't have the basic toxicology knowledge in order to apply for an investigational new drug application from the Food and Drug Administration.

So this is where the huge worry comes in, is that we have hundreds and thousands of young people in the US and around the world who are experimenting with these new drugs, many of whom become addicted and dependent on them, and we don't even know the basic toxicology data so that I could run a study with doctors and nurses and very tightly controlled circumstances.

So it's very frightening, because we have a huge experiment going on in our country and around the world with these new drugs, and the adverse events that occur are very severe, including death.

To my knowledge, I have never seen a cannabis death where it was due to the heart stopping or breathing

stopping, where the drug actually killed the person. Certainly, they can die in car accidents when they are driving impaired under cannabis, and they could have hallucinations, and they could die from jumping off a building when they think they can fly.

But we now know that these novel psychoactive substances, that are up to a 100 times as potent as cannabis, actually produced death, as do the synthetic cathinones and other novel psychoactive substances.

So we have developed in my lab, which we didn't do at all, three-and-a-half years ago, we didn't do any of this, now we have a very good method for determining the metabolism of these novel psychoactive substances. And we have learned along the way, but we have applied both liquid chromatography, tandem mass spectrometry, to understand the half-life of the drugs, that's with human liver microsomes. That's critically important, because it enables us to design our experiments with human hepatocytes, live human liver cells, appropriately.

And we learned the hard way, we didn't do the human liver microsomes. We were told by the pharmaceutical industry it wasn't necessary, but we learned very quickly that as the clandestine chemist started designing these drugs, some of them were really prodrugs, and they very rapidly changed. And so unless you know that by doing the human liver microsome work, you could design your experiments with the live liver cells incorrectly.

Now, most of this work that you will see in the literature is done with human liver microsomes, but the disadvantage of that is that in the human hepatocytes, the cells are actually reproducing the actual environment. And so the drug has to get into the cells, and the metabolites that are produced are actually produced in similar ratios to what we have in human urine.

Whereas, if you used human liver microsomes, they can produce the metabolites, but there isn't any relationship to what you might actually find in a human urine sample. So very important to do the human hepatocyte work so that we can focus on the most important metabolites.

Now, why do I say metabolites, and that's because for the synthetic cannabinoids, very little to none of the parent drug is present in the urine. And so unless you know what metabolites are produced, you can't measure these, and this is the critical role we are playing.

We are determining the half-life of these drugs; that's critical for interpretation of drug-drug interactions,

understanding how long the person may have symptoms, and how long you might be able to monitor the drug. And the human hepatocytes will tell us what are the major metabolites that not only laboratories around the world need to focus on, but also standard manufacturers.

So one of the biggest problems is we don't have the standards and so by doing this work we tell the commercial standard manufacturers, focus your attention on these. They don't want to spend the money on 25 different metabolites not knowing which are the most important, but if you can tell them, these are the key metabolites, then they can do that work.

So this special issue about mass spectrometry, we have taken a brand-new application of mass spectrometry that we have used for years, and we are using both tandem, LC-MS/MS, and we are using high-resolution mass spectrometry.

And my colleague, Dr. Diao, is going to talk about how we use that. You need a very sophisticated instrument like a QTOF instrument that has both the chromatography and the high-res mass spectrometry to determine these metabolites.

And it's like taking a 2,500 jigsaw puzzle, with all those pieces and throwing them up in the air and letting them come down and trying to put that back together without a picture. And that's what we are doing when we do these metabolism of these drugs to try to determine how your body, the human body, will metabolize them and what metabolites they will produce.

Bob Barrett: Well, you mentioned of course our special issue about the importance of mass spectrometry to the clinical laboratory, talk about how mass spec enabled you to determine human metabolism of two new synthetic cannabinoids: THJ-018 and THJ-2201.

Dr. Marilyn Huestis: Well, the first I talked a little bit about the tandem LC-MS, that's critical. What we do is measure the disappearance of the parent drug. And in this particular issue, we are talking about these two new synthetic cannabinoids that are becoming problematic: the THJ-018 and 2201.

But we are using the same techniques on many new drugs that are just released, and we only do this work if nothing is known about the metabolism. If for some reason someone else has been able to determine some of this information, we don't do it. But the real key here is the high-resolution mass spectrometry, and that's what exquisitely to the fourth decimal place enables us to differentiate these molecules that are very closely related.

So we use two primary mass spec tools: tandem mass spectrometry and the Q-TOF, the chromatographic time-of-flight high resolution mass spectrometer.

Bob Barrett: Well, now let's turn to the first author of this article Dr. XingXing Diao who is a postdoctoral fellow at the Huestis laboratory.

Dr. Diao, what are the analytical challenges to determining the best markers for clinical and forensic laboratories to determine new synthetic analogs?

Dr. XingXing Diao: Yes, our lab in National Institute on Drug Abuse started on the most new synthetic cannabinoids, show the reference standard for the metabolites are currently not available. So we do not know the exact position of the metabolites.

Also the synthetic cannabinoid analogs, sometimes have very minor changes, for instance the only difference between THJ-018 and THJ-2201 is the terminal fluorine. So it is possible that they generate the same metabolites. Also the synthetic cannabinoid parents is highly diluted in the urine. As Dr. Marilyn Huestis said, they are instantly metabolized so they have very low concentration. We have to identify the specific metabolites and the proper strategy to confirm their consumption.

Presently we used some encylic software to predict the possible metabolites. It has helped us to get a general idea over the metabolites information. However, it has some limitation, it does not predict the Phase II metabolites, it only predicts the cytochrome P450 catalyzed interactions.

Dr. Bob Bergen: Well doctor, what did you find out about the two synthetic cannabinoids, THJ-018 and THJ-2201 metabolism?

Dr. XingXing Diao: Yeah, for THJ-018 and it goes with hydroxylation on the side chain further carboxylation, glucuronidation. The dealkylation and the dihydrodiol formation were also observed.

For THJ-2201 we observed oxidative defluorination, further carboxylation and glucuronidation as a major metabolic pathway. However, we also observed uncommon metabolic pathway in THJ-2201. It is glucuronidation after N-oxide. This uncommon pathway was not observed in any previous synthetic cannabinoid.

Bob Barrett: How will these data be used by laboratory professionals to identify use of THJ-018 and THJ-2201?

Dr. XingXing Diao: We identify the major metabolites for THJ-018 and THJ-2201. They share two major metabolites and they also have their own specific metabolites. So the clinical laboratory can incorporate the metabolite information into their screening method and target the consumption of THJ-018 or THJ-2201.

We can also use a data to link the adverse events to them and educate the public about their dangers and toxicity. Also the manufacturers, can focus there synthesis efforts on the best target metabolites.

Dr. Bob Bergen: And finally Dr. Huestis, back to you, we have talked about this before, it seems like a never-ending problem. What do you think is the future for clinical and forensic laboratories for monitoring synthetic cannabinoids and other novel psychoactive cannabinoids?

Dr. Marilyn Huestis: Well, I believe first of all that it is not going to go away, that this is going to be a continual problem for laboratories around the world, and it's going to be something that in the future all laboratories are going to have to be able to do. Right now, really, reference labs and specialty labs are doing this work, but I think in the future it's going to be necessary for all labs to address this problem.

And perhaps you send out to laboratories, reference laboratories, but it's going to be critically important that every lab has the knowledge and the understanding about these compounds. I believe having started here in my own lab, the very first method we used tandem mass spectrometry and we identified 29 markers for synthetic cannabinoids, and that was by far the most that were included in any method.

Other methods might just address JWH-018 or they might just address JWH-018 and may be 2201, we had 29 different markers. But within months that method was out of date, and our next method was 53 analytes, and you quickly realize that you just can't go on in that way.

And so what I believe the futures going to be for the labs is to have high-resolution mass spectrometry in their laboratory, and the cost for this equipment is decreasing tremendously. I think that you are going to have to have highly trained operators, and that you will use a non-targeted approach. In other words, you are not going to typically do what we do now, which is include 20 or 30 or 40 analytes in a method.

You are going to have to be looking for any analytes, and we recently published another manuscript that was non-targeted SWATH screening. Every instrument manufacturer

has a different name and has the capability with their high-resolution mass spectrometry, but it's going to be non-targeted. That means all of the ions are going to be collected and all of the spectrum will also be collected.

And you will then have to go in search of not only compounds that you know about but perhaps you will have data that is available to you in the future if a new drug you learn about it, you can go back and you can look at your other chromatograms to see whether or not that may have been present, or specific signatures that might mean you are going to investigate compounds that have these specific ions.

So I think we are going to have to be facing this for the future in front of us and we are going to need high-resolution mass spectrometry to address this problem. And it's absolutely key, as Dr. Diao said, that you have to be able to link the adverse events that occur, the death, the strokes, the myocardial infarctions, the kidney damage, all of these terrible adverse events have to be linked to specific compounds.

And as we also told you is when we look at this work for instance, the JWH-018, the AM-2201 that were first generation synthetic cannabinoids, only differ by a small molecular change to produce the THJ-018 and the THJ-2201.

And so we have been able to offer ways to differentiate these compounds from each other and there are analogs, and that’s especially important when it comes to the legality of these substances.

In our country we have an Analog Law, that says, all compounds of these classes, if they are, for instance cannabimimetic, they bind to cannabinoid receptors, are illegal. But the manufacturers are trying very hard to make changes significant enough that that Analog Law will not be applicable.

And unfortunately, we don't have the case law yet that says whether or not these compounds will stand up as illegal.

So that’s in our country where we have the Analog Law, but in other countries like Germany or Sweden, they have to lift the full chemical structure of every compound that is illegal or scheduled. And in those cases, it's critically important that you can differentiate between analogs that differ very little because one may be illegal and one may not.

So in our work we have really harnessed the energy of mass spectrometry to address this critical problem, and that’s why

we were so happy to be included in this special issue because it really shows a new application of a powerful tool for our laboratories.

Bob Barrett: That was Dr. Marilyn Huestis, Senior Investigator and Chief of the Chemistry and Drug Metabolism section at the National Institute on Drug Abuse.

She was joined by her co-author Dr. Xingxing Diao from her laboratory as our guests in this podcast from *Clinical Chemistry* on synthetic cannabinoids. Their research appeared in the January 2016 issue of *Clinical Chemistry,* a special issue devoted to mass spectrometry and the clinical laboratory.

I am Bob Barrett. Thanks for listening!