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Xylazine Pharmacokinetics in Patients Testing Positive for Fentanyl and Xylazine

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Guest: Dr. Bridgit Crews from Barnes-Jewish Hospital and the Department of Pathology and Immunology at Washington University School of Medicine in St. Louis, Missouri.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

Xylazine. You likely have heard of it on the news and may even test for it in your clinical laboratory. But what is it, and why has it suddenly burst onto the scene in clinical laboratory and medical toxicology circles?

Xylazine was initially intended to treat hypertension, but due to significant side-effects, was never approved for human use. Currently, its legal use is limited to veterinary medicine, where it's used as a sedative. More recently, it's been found in illicit fentanyl, cocaine, and heroin, with close to a 300% increase in xylazine detection in fentanyl-associated overdose deaths between 2019 and 2022. In part, because xylazine is unavailable for regulated clinical use in humans, relatively little is known about its metabolism and window of detection.

Animal studies have shown half-lives as short as one hour, but this varies by species, making it difficult to predict whether animal studies translate to humans and whether coingestion of fentanyl affects xylazine metabolism.

A new research article, appearing in the February 2025 issue of *Clinical Chemistry*, fills this knowledge gap by establishing the xylazine half-life in patients using fentanyl mixed with xylazine. In this podcast, we are pleased to speak with the article's senior author. Dr. Bridgit Crews is the Medical Director for Toxicology and Mass Spectrometry at Barnes-Jewish Hospital and an Associate Professor in the Washington University Department of Pathology & Immunology at Washington University School of Medicine in St. Louis, MO.

So, Dr. Crews, let's start with the basics. Just what is xylazine?

Bridgit Crews: Yeah. So, it's a medication that's really used in veterinary practice. It's used to sedate animals, either for surgery or to relieve pain. It's a non-opioid sedative and it's a muscle-

relaxant. So it's not been approved for human use, but it's been found in uncontrolled illicit drug supplies.

It's been around for a really long time, decades, but the reports of detecting it in illicit drug supplies have increased sort of starting around 2008 when it was first identified in Puerto Rico. So since then, and recently in the United States, there have just been more-and-more reports of detecting it in drug supplies, in postmortem cases, and in patients. Its use in veterinary medicine is for large animals such as horses.

And so, the list of drugs that contain xylazine are sometimes anecdotally referred to as "tranq" or fentanyl mixed with xylazine is referred to as "tranq dope." And it's… so therefore, in humans, or humans that use fentanyl mixed with xylazine, they really use it sort of in the same manner that they would use fentanyl, so they can inject it, or smoke it, or insufflate it, snort it, or inhale it, or even swallow it like a pill if it's in that formulation.

Bob Barrett: Do people who use these drugs know that they contain xylazine?

Bridgit Crews: We really only have anecdotal evidence of whether or not people know they're being exposed to it. It seems like people are reporting that they do recognize that the drug that they're taking contains xylazine or contains tranq. Like maybe they know based on like how they feel or they suspect that it has xylazine. There was actually a study published that suggests that people don't necessarily seek out drugs that contain xylazine, so it's not like they're trying to take it. But really, in reality, the only way to definitively know if the drug has xylazine in it, is to either test the drug, which is available in some drug checking programs, but those aren't like widely available in the US.

So, the only way people could know that they're getting it is just based on how they think they feel after they take it, unless they're able to test their drugs for xylazine, which is not commonly done.

Bob Barrett: What is known about the effects of xylazine in humans?

Bridgit Crews: So, the primary mechanism of action of xylazine is agonism of alpha-2 adrenergic receptors. There may be some other targets that it interacts with. So, most of what we know about its effects comes again from animal studies or from single case studies of persons who overdose on just xylazine, maybe they accidentally inject themselves with the veterinary medicine or they purposefully take it. And then, but most of what we know about people who are exposed to xylazine mixed with fentanyl just comes from those observations or those anecdotal reports. So, we don't have a lot of

information. So, the self-reports are that they feel very sedated, they sleep a lot more. Some people will claim that they black out a lot more after they are exposed to these drugs that contain xylazine.

One of the most concerning observations is that people have found it's associated with these severe skin ulcerations. So there seems to be an association when people are repeatedly exposed to fentanyl mixed with xylazine that they develop these severe necrotic wounds that in some cases can involve like the deep tissues or even the bones. And it's not really clear what the underlying mechanism is there or how to potentially even mitigate that risk, other than not using the drugs that contain xylazine.

Bob Barrett: Now this study focused on pharmacokinetics of xylazine in persons who use fentanyl. How is this accomplished, and well, what were your main findings?

Bridgit Crews: Yeah, so pharmacokinetics just basically is how the drug moves throughout the body, how is it absorbed, how is it distributed in the different compartments of the body, how is it metabolized, and then finally eliminated or excreted?

And so, when we were first thinking about the study, there seemed to be a lot of information out there that xylazine had an incredibly short half-life. It was repeatedly stated that it had a half-life of less than an hour. But this was just based on a subset of animal studies. And if that was true, if xylazine has such a short half-life, then what's the explanation for these reports of like really prolonged sedation in individuals? Because this is what people were kind of concerned that it would potentially exacerbate the sedation. So, you would treat someone with an opioid overdose, but then maybe they wouldn't respond as expected because there was xylazine onboard. And so, how would you explain that sort of like prolonged action if it had such a short half-life? And there's different ways it could be explained and all go together, but it's just there was limited information.

And so, one of our goals was try and just gather some information on how long we might expect to be able to detect xylazine in persons who are using fentanyl mixed with xylazine and look at how quickly the xylazine was being metabolized, what it was being metabolized into, and how quickly it was being fully eliminated.

So, in order to do this, first we had to identify individuals who are exposed to fentanyl mixed with xylazine, and luckily, we were already testing for xylazine in the urine of patients who are positive for fentanyl in our lab, and so, we're able to identify them that way. But then, we had to identify remnant blood samples from those patients and be able to collect serial

samples from them, samples that were collected for clinical testing, but then were being ready to be discarded. We were able to find those and save them before they were thrown away.

And then we set up a method to quantify xylazine. We were able to do that because there was a standard available, and there was also a standard available for one of the metabolites, which was thought to be a major metabolite in urine, was the hydroxy metabolite. So, we're able to set up targeted methods to quantify both of those analytes.

But then, we wanted to see if there was any other prominent metabolites we could identify, and perhaps look and see if any of the metabolites were detectable for a significantly longer amount of time than xylazine. We found, or we calculated, that xylazine had a terminal elimination half-life of around 12 hours in the patients that we collected, in the patients who were exposed to fentanyl mixed with xylazine, but there was like a pretty wide range. The shortest half-life we calculated was 6 hours and the longest was 21 hours. And we excluded a lot of patients. We excluded patients who had known renal insufficiency because it's thought that xylazine undergoes both hepatic and renal routes of elimination. And then we were also able to identify many of the metabolites that were previously characterized in rats.

So, there were two publications that were published a little over a decade ago that we really relied on heavily in order to be able to do this.

So that was a publication by Mutlib and another one by Meyer, and they actually did an extensive structural characterization of the xylazine metabolites that were observed in rats and some other animals, and they actually published the full scan tandem mass spectra of the metabolites. And so, we were able to use that and identify the same metabolites in our patients' blood and we confirmed that by matching the product ion spectra to what they published and it matched really well. And then we set up multiple reaction monitoring experiments to sort of look at the most intense transitions for all of the metabolites we were able to detect. And then we quantified of course xylazine and the hydroxy-xylazine. We found the high signals for sulfone-xylazine and oxo-xylazine and that was kind of a little bit different than I think what people were expecting based on the animal studies.

Bob Barrett: So, what was unique about this approach?

Bridgit Crews: Well, I think how we use the remnant samples is not a common approach. And when we started the study, I didn't really know if we were going to get any interpretable information. I mean, we're kind of just taking patients with

a wide -- you know, there's a lot of variation among the patients.

We didn't know the dose of xylazine. We didn't know when they last took the xylazine. We didn't know how they were administering the xylazine to themselves. Are they smoking it or injecting it? So, we just kind of like assumed those were all just going to be variables.

We didn't really know anything other than like the time points that the specimens were collected and we kind of arbitrarily set our first specimen collection to like the first time point, time 0. And then we had a little bit of information we could get from the patient chart, like certain diagnoses or certain laboratory values, but a lot of these patients, there wasn't an extensive work up for them because they were just coming to the emergency department to get limited care and then leaving. It was quite difficult to collect enough samples to really have a long enough timeframe to measure how quickly the xylazine went down.

So, I think that was kind of like one unique feature. We didn't apply any sophisticated statistics to do it, at this point in time, but I think in the future hopefully we'll do that.

Bob Barrett: And were there limitations to this approach?

Bridgit Crews: Yeah. So, it really was a brute force approach. I think that that was a limitation in how we're going to like do it a little bit more elegantly in the future. So, we're trying to figure out a better way to sort of identify and collect the appropriate types of specimens so we can sort of expand and look at more variables. And then there's a lot of information we're missing obviously. And so, we're trying to figure out like exactly what information would be relevant to collect and how we're going to go about collecting that.

And then there's limitations to the data itself. I mean, we had to exclude a lot of patients and that's detailed in the paper, and obviously the data is not going to necessarily apply to those subset of patients. Analytically, we didn't have standards to quantify a lot of metabolites and so we're just basing it on relative abundance of the signals that we saw and that could be misleading, potentially if there's differences in the ionization efficiency of the metabolites or enhancement or suppression at different chromatographic times. There's metabolites, for sure, that we didn't focus on and detect. We didn't look at any glucuronidated metabolites in the blood.

So, I think if we were able to get standards, we would definitely go back and want to quantify and confirm that the abundances we reported were consistent with the actual concentrations.

Bob Barrett: Well, finally, Dr. Crews, how can these types of studies impact care of patients who use drugs that are adulterated with xylazine?

Bridgit Crews: So, we're really limited right now on how we can study drug adulterants. It's not really ethical to do controlled study on these drugs that are not approved for use in humans and never have been, and particularly like we're seeing a lot of mixed exposures, certainly not ethical to be mixing a bunch of drugs together and looking at how it affects patients. So, I think we're seeing more and more of this in the uncontrolled drug supplies like fentanyl mixed with xylazine and stimulants, and then novel opioids and other psychoactive substances as well.

So, I guess, my hope is that we can do these types of studies and learn something that can be used to prevent the harm these drugs are causing, so we can provide more effective care and treatment, but I think there's definitely a lot more work that needs to be done. I think we have a little bit better handle now on how long we can detect the xylazine in blood, what the major metabolites are.

I know there's multiple groups who are doing more retrospective cohort studies to look at clinical outcomes, and I think a lot of people are interested in gathering more information on how the mode of administration or the frequency or size of the dose can affect the exposure and clinical outcome.

I think some of the next steps for us might be to compare the relative concentrations of xylazine and fentanyl in the patients that we can measure in the blood, and then, also potentially look at whether any of the major metabolites have any activity.

Bob Barrett: That was Bridgit Crews from Washington University School of Medicine in St. Louis, Missouri. She served as senior author of a new research article defining the pharmacokinetics of xylazine in the February 2025 issue of *Clinical Chemistry*, and she's been our guest in this podcast on that topic.

I'm Bob Barrett. Thanks for listening.