Episode 35: Xylazine and Other α2 Agonists - Clinical and Forensic Perspectives

Transcript

Hi, everyone. Welcome back to the Tox Lab. I'm Rebecca. I'm Rob.

This week, we're going to be taking another look at some recent papers looking at xylazine. We first talked about xylazine, episode one, I think. Yeah. We've possibly mentioned it a couple of times since then.

It's been a bit of a journey, hasn't it? It feels like a long time ago now. It really does. So xylazine is an interesting drug because it's an alpha-2 agonist.

And alpha-2 agonists are actually a fairly interesting class of drug. They stimulate the natural feedback loop that adrenaline leans on. And effectively, they act a bit like a bouncer in a bar. They say, come on, you've had enough adrenaline now, calm down.

And they lead to sedative effects. So reductions in central nervous system activity, reductions in breathing, reductions in effectively the opposite of the fight or flight response. And xylazine is interesting because it's a drug that is commonly used in veterinary medicine, but it started to see emergence as an adulterant in opioids. So mixed in with fentanyl in the US and sometimes mixed in with heroin in the UK.

So we're going to take a bit of a look at some recent publications looking at xylazine and maybe have a bit of a catch up on where we're at with that. Before we jump into today's episode, if you want to stay up to date with our content, you can find us on Instagram at @the_tox_lab. We also have a LinkedIn page. And for those of you who don't use social media, but still want to get in touch, you can email us at thetoxlab@gmail.com.

So Rebecca, xylazine, we've got two fairly hot off the press research papers, haven't we? Both looking at a couple of unique aspects of xylazine. Why don't you introduce us to our first paper? So the first paper is looking at a rat model of co-self administration with the aim of investigating how xylazine affects heroin consumption, motivations and reinstatement behavior.

It also takes a look at the influence on locomotor activity and naloxone precipitated withdrawal symptoms after self administration. So with the title, I thought, how on earth do you get rats to self administer heroin? And essentially they had active and inactive assigned nose pokes, and this would administer drug infusions at a fixed rate. Once they self administered, there would be a five second cue of light before 20 seconds of darkness when no reward could be earned.

So different rats were separating to different groups. So you had heroin alone, xylazine alone, saline as a control, and then heroin and xylazine co-administration. And it seemed that the heroin group self administered more than all other treatment groups after a day of abstinence. They did some progressive ratio experiments, so a delivery schedule ratio increased following each successfully earned infusion.

Basically, I think the dose is increased and the group that were taking heroin alone self administered significantly more infusions than all other groups. And the heroin xylazine group administered

significantly more infusions than both saline alone and xylazine alone. And animals exposed to heroin exhibit the highest breakpoint. So the highest number of self administered infusions.

And that was significantly higher than the co-administration group. So xylazine and heroin. So after five days of abstinence, the rats were returned to their original operating chambers for 10 days where queues or rewards were not presented to kind of remove any drug seeking behaviour. And there were no significant differences in either of the groups, suggesting successful extinction of seeking behaviour.

So this represented a kind of abstinence model following use of the drugs. Yes. On day 15, they introduced drug associated cues and house lights, but no drugs were available to the rats. And in the animals just taking heroin, there was a significantly higher response for cues than saline.

And the heroin xylazine co-administration group showed an even greater seeking behaviour than the heroin group alone. So they were suggesting that xylazine enhanced heroin's cue induced reinstatement behaviour. So to give you some context as to why this paper actually caught my eye, xylazine is a drug which has been increasingly found as a contaminant in fentanyl mixes in the states. We've seen it a reasonable amount in the UK, often mixed with nitazenes.

But there's been a bit of a conundrum with xylazine in that the concentrations of the drug that we are seeing post-mortem is quite a bit lower than the concentrations normally associated with sedation and other CNS effects. And it's been a bit of a mystery to me what the xylazine is actually doing. I think episode one, we looked at a paper, didn't we, where there was some suggestion that xylazine was attenuating opioid receptor behaviour. But I think the key finding for me with this paper that really caught my attention is that actually xylazine seemed to make the rats undergo more relapse.

Studies like this do make me glad I'm not an animal researcher. Yeah, because there's a lot going on here, isn't there? And it's quite hard to sort of unpick some of this. But that really, for me, was quite key finding.

Were there any other key findings that struck you? They did do some experiments looking at locomotor effects as kind of a marker of sedation, and xylazine didn't show any sedative effects. I think that's another reason, actually, that this paper caught my eye, because again, it's using almost a sub-therapeutic or sub-sedative dose of xylazine. This is still early data, isn't it?

This is only one study. And obviously, we don't know how well this translates to humans. But it does pose the potential question, doesn't it? Is the inclusion of xylazine in street opioids maybe not having so much a sedative effect or an enhancement effect, but actually potentially more of a reinforcing effect?

Hmm, potentially. It is important to note that all of the rats in the study were male. So I believe it's been quite a long time. But that first paper we talked about on xylazine back in our very first episode, there were some sex-specific differences in xylazine's effects.

So it'd be interesting to see whether in female rats the same thing happens or whether there are subtle differences. Yeah, that's a completely fair point. It was a long time ago, but I do remember us talking about sex differences in xylazine and fentanyl, possibly. Might have just been xylazine on its own.

It's a long time. OK, so that's one paper looking at xylazine. Why don't we look at our next one? So this next paper looks at potential treatments for fentanyl and xylazine overdoses.

And this is really important, right? Because naloxone is a really, really effective treatment against opioid overdose. But crucially, naloxone may not reverse xylazine. There's some debate, actually, isn't there, as to whether naloxone does have some effect on xylazine?

But it certainly isn't its primary mode of action. So this is potentially really important research. So in 2023, 30% of all fentanyl powder seized by the DEA contains xylazine. Fatal drug overdoses involving xylazine are also on the rise.

So in 2018, they accounted for 0.03 per 100,000 and have increased to 1.06 per 100,000 in 2021. Ninety nine point one of these fatal overdoses involving xylazine were co-administration with fentanyl. A xylazine overdose can cause effects such as respiratory and CNS depression, hypotension, bradycardia, hypoglycemia and hypothermia. So this paper looks at a combination of naloxone to treat the fentanyl overdose and atipamazole, which is an alpha-2 antagonist.

And this has been used in veterinary medicine to reverse sedation caused by dexmedetomidine, which is also an alpha-2 agonist. And it has been shown to reverse sedation, hypotension and bradycardia in humans. So this paper was kind of like a preclinical trial designed to assess the safety and efficacy of this treatment combination in the reversal of a fentanyl xylazine intoxication. So naloxone works by displacing opioids from their opioid receptor.

This drug works by effectively doing the same thing on the alpha-2 receptor. So they did some doses studies initially to work out what dose of fentanyl and xylazine would cause sedation, and they settled on 0.1 milligrams per kilogram of fentanyl and 20 milligrams per kilogram of xylazine, which produced an intense and moderate sedation in rats. Intense and moderate. Yeah, that was their wording.

Interesting. Mm. So in rats, given fentanyl only, atipamezole showed no effect on consciousness, heart rate, blood glucose, but did show a dose dependent reduction in the effects that xylazine has on these assessments. 0.1 milligrams per kilogram of atipamezole showed near maximal reversal of xylazine-induced sedation.

But 1 mg per kilogram was needed to attenuate the bradycardia and the hypoglycemia. Interestingly, both these doses didn't quite completely reverse the hypoglycemia, but giving this drug in the absence of xylazine, you do see a mild temperature reduction, which I thought was quite interesting. That's interesting. Yeah.

So again, they also looked at locomotor activity in these rats by giving them methamphetamine. So fentanyl attenuated meth-induced locomotor activity within about 10 minutes. Xylazine hastened and intensified the sedation, resulting in significant decreases in meth-induced activity compared to fentanyl alone. When giving naloxone and 0.1 mg per kilogram of atipamezole, the total distance travelled was significantly increased.

In other words, it effectively reversed sedation. Yeah. They also looked at skin lesions. Xylazine has been known to cause these really horrendous skin lesions.

And seven out of the eight rats who were exposed to xylazine had skin lesions at the injection site. But rats who weren't exposed to xylazine didn't have any of these effects. And the skin lesions, things, fairly interesting phenomena, isn't it? We think it's to do with peripheral vasoconstriction.

Again, I'm not sure if that's been confirmed or whether that's just currently a working hypothesis. Interestingly, three rats who were exposed to atipamezole experienced red tears. Interesting. Which I think might be a known side effect of that drug.

OK. Blood, presumably, or not just red. It's just described as red tears. I'm not entirely sure what the origin of the red is.

Interesting. And xylazine did show a small but significant effect on lymphocyte and basophil counts and lymphocyte and neutrophil percentages. So they proposed this drug as a potential reversal agent for the combination of with with naloxone as a combination reversal agent. And their data showed

that it was better than naloxone alone.

Yes. I think we want to drill into the methamphetamine data as well, don't we? Because that was a bit of an adverse effect. If you've got an adrenergic stimulant present and then you block your alpha-2 receptor, they saw an increase in stimulant activity.

Yeah. So again, for context, it's not uncommon for fentanyl containing xylazine or heroin containing xylazine to be mixed with stimulant drugs, which is why the authors actually tested this hypothesis with methamphetamine. Possibly you'll see the same thing here with cocaine co-use as well. So I guess there is that added risk that if you factor in a stimulant to the mix, there could be additional adverse effects to potentially treating the xylazine.

And I think this is the problem with a lot of these potential treatments is generally speaking, people aren't just going to be on the drug that you're treating them for in a sort of overdose situation. As we always talk about, mixed drug toxicity is really common. So what one treatment might do to reverse one kind of overdose might make something else worse. Yeah, that's completely fair.

So I guess the next question, it seems effective in rats. And it has got some use in humans, hasn't it? You said it's been used to reverse medetomidine. Yes, yeah, it's reversed the sedation and the bradycardia associated with medetomidine.

So it's possibly actually already approved for use in humans. Is a human study warranted next? Potentially, it's quite hard to get ethical approval for those sorts of things. I think all you could do is use it as a treatment arm in an ED situation where you've got a lot of xylazine.

I don't think we could start doing fentanyl and xylazine administration studies. No, I can't see that ever getting approved. Has potential, though, doesn't it? Especially if it is already approved for use in humans in some other context.

Yeah, that tends to make regulatory approval quite a bit easier. Now, there were a couple of points I did think, actually, with this study. Like the first one, they only use male rats, which again, possibly given the sex differences, could have influenced the outcome a bit. And probably we should move towards testing with female rats, too.

Yeah. I think also in this paper, they talked about a rat coma scale to describe the levels of sedation. Yes, they did. But I think that might have been made up.

I don't think it's a standardized scale. No. So again, possibly some bias in there, but I appreciate sometimes these things are hard to quantify. OK, so that's two papers looking at xylazine.

Shall we move on to something else? So the next paper we're going to look at is not xylazine, but it is looking at medetomidine, which, as we've mentioned already, actually, is a similar drug. Rebecca, introduce us to the paper and medetomidine. So this paper is looking at medetomidine and its enantiomers across many different biological samples.

medetomidine is 200 times more potent than xylazine and is thought to have a tenfold greater selectivity at the alpha-2 adrenergic receptor. It exists as two enantiomers. dexmedetomidine is approved in human settings and dexmedetomidine and a mix of dex and levomedetomidine are approved for use in veterinary settings. dexmedetomidine is active at the pre- and postsynaptic sites in both the central nervous system and the peripheral nervous system, whereas levomedetomidine is relatively inactive and not prepared or used alone.

So dexmedetomidine does all the heavy lifting. And we've talked about enantiomers before, but if you are new here and haven't heard us talk about enantiomers, we did an episode a few weeks back, didn't

we, looking at methamphetamine enantiomers. So go check that out. So this paper looked at 100 authentic biological specimens, 70 of which were coming from emergency departments, 30 which were coming from medicolegal death investigations between May 2024 and January 2025.

All samples were screened using LC-QTOF-MS. And the majority of samples came from 14 different states across the US. Pennsylvania was the main contributor. And there were samples from Canada and the UK as well.

So medetomidine is a drug very similar to xylazine and in response to the xylazine emergence in the US and across the world, actually, some controls have been put in place to try and make it harder for people to get hold of. And as a result, medetomidine has possibly, although not conclusively, started to take its place as an alternative to xylazine. As Rebecca pointed out, it's quite a bit more potent than xylazine, gram for gram. So in cases where demographics were given, the majority of individuals were male aged between 21 to 74 years with a median age of 50.

They quantified the levels of medetomidine in both post-mortem samples and those collected anti-mortem and concentrations ranged in post-mortem blood from 0.1 nanograms per millilitre to 32 nanograms per millilitre. And 93 percent of these samples contained fentanyl and xylazine. In the anti-mortem samples, concentrations ranged from 0.1 to 16 nanograms per millilitre and only 76 of these contained fentanyl and xylazine. Quite a chunk there, though, that actually did have xylazine mixed in with them.

So it's not just replacing xylazine. It seems to be coming alongside xylazine. And in the post-mortem samples, xylazine was found at concentrations between 0.1 and 100 nanograms per millilitre and in the anti-mortem samples between 0.1 and 60 nanograms per millilitre. So generally, medetomidine was found at lower concentrations than xylazine and it's thought because of its increased potency.

But in 21 percent of the cases, medetomidine was found at a higher concentration than xylazine. Interesting. I'm not sure what the clearance kinetics are, whether one's got a longer half-life or things like that. That could influence that as well.

Possibly. Either way, though, a reasonable amount of medetomidine present and often present with xylazine, which is concerning. They did also look at medetomidine's metabolite, 3-hydroxymeditomidine, and this was rarely detected in blood, but was found in urine in about 38 percent of cases. medetomidine was also found alongside other drugs, including a traditional stimulant such as cocaine in 35 percent of cases.

Parafluorofentanil was found in 34 percent of cases. It was also found alongside NPS drugs such as bromazolam, metonitazene, and N-desethyl isotonitazene. So, nitazenes are very potent opioids. We've talked about a few times on this podcast before.

We've talked about bromazolam as well. So looking at the two different enantiomers, dexmedetomidine and levomedetomidine were found together in 90 percent of submitted blood and urine samples, which covered 65 non-fatal cases and 25 fatal cases. In the remaining 10 percent of cases, dexmedetomidine was found alone. In 40 percent of these, no fentanyl was detected.

And in 80 percent of these, no xylazine was detected. And they believe that only dexmedetomidine was found in these cases because it was administered as part of their medical treatment upon admission. Yeah, it's not a drug we tend to use in the UK very often, but I have seen it in the US used as an ED drug. So most of the enantiomer profiling suggested this was veterinary origin?

Yeah, I think they were thinking either diverted veterinary medicines or clandestine lab production. Makes sense. And you can buy medetomidine on grey market websites. So currently not very well

controlled, if at all.

They did also look at the effects of medetomidine. So non-fatal effects included bradycardia, which was seen in 56 percent of cases. You can get prolonged sedation, drowsiness. In 20 percent of cases, naloxone administration had no effect.

And hypotension and hypotension were also seen. Hypo and hyper. Yeah, both were reported. Yeah. Interesting.

So what I really appreciate about papers like this is that they do provide a really good base for interpreting levels. So if we were to quantitate this drug seen in our lab, you can kind of get an idea of whether that level is consistent with someone who's still alive or whether it's consistent with post-mortem levels. And that could be a really valuable tool for a drug that you've not seen and you don't have experience with yourself. Yeah, it's really important, isn't it?

And especially really appreciate this paper coming out now because this is a relatively new emerging drug. And actually, they've done a really good job of getting this paper out. They're quite quick. It could potentially be really useful if we start seeing medetomidine in the UK.

It sounds like potentially it already is, given that they've tested some samples from the UK. This is another case of potency escalation following control, isn't it? xylazine was a thing. Controls have been vaguely put in place.

Try and stop the diversion of veterinary medicine and so on. And now we've seen a move towards medetomidine, which is more potent. I do worry that the concentrations we were seeing of xylazine were actually really low, aren't they? And medetomidine being even lower.

I do worry that we might start missing some of these cases. Are labs ready to detect medetomidine? Clearly, it's going to be clinically relevant to some degree in some cases. I think there is a risk that certain instruments won't be sensitive enough to pick up the really low levels of medetomidine if it's present at even lower levels than xylazine is.

And I know some labs are struggling to pick up xylazine in their case work. There is a real risk that we are going to be missing some things. Yeah, I think that's completely fair. The point about the metabolites is true as well, actually, particularly with xylazine.

You don't always see metabolites, particularly in blood. I guess the obvious question is, should moves be made to try and control medetomidine? But then will we just move again to something even worse? I think that's always the risk.

You block something and then a whole host of other drugs crop up in its place to fill that gap in the market. A common theme, I feel. So let's move on to our final paper. Now, this is looking at yet another alpha-2 agonist drug, tizanidine.

So take us through this paper. So just a heads up before I jump into this paper, there are mentions of suicide in this. So if that's something that you don't want to be listening to, then please click off this episode. So tizanidine is another alpha-2 adrenergic receptor agonist.

It's used as a skeletal muscle relaxant. It is quite short acting. It can both prevent skeletal muscle spasms and spasms with smooth muscle. So this paper looks at 18 post-mortem cases involving tizanidine.

Samples were screened either using an orbitrap or a cutoff high-res mass spec. And tizanidine was quanted using LCMS. Cases were divided into suicide where tizanidine was implicated, accidental

deaths where tizanidine was implicated and incidental findings where tizanidine was not implicated. So eight cases of suicides and concentrations of tizanidine range from two micrograms per litre to 38 milligrams per litre.

Four accidental cases where concentrations range from 0.17 milligrams per litre to 1.5 milligrams per litre. And six cases of incidental findings where tizanidine was found at 5.8 micrograms per litre to 1,970 micrograms per litre. All of these cases did involve other drugs present at toxicologically relevant concentrations. The majority of individuals were female and aged between 42 and 69, with the average age of 54.

Quite big ranges of concentrations detected in those groups, wasn't there? It does appear to be influenced by some, I don't want to say outliers, but some very, very high concentrations. So when you look at the median concentrations across the three groups in suicide, the median was 0.77 milligrams per litre accidental was 0.89 milligrams per litre and incidental was 0.035 milligrams per litre. But yeah, clearly, particularly in the suicide group, there was one or two really, really high concentrations.

Yeah. And also it's important to note with this dataset that it's quite hard to determine whether something was intended to be a suicide or whether it was accidental ingestion. A lot of this was due to seen evidence. So the majority of the suicide cases had notes or evidence that suggested suicidal intention.

The two cases involving the highest concentration of tizanidine also involved other substances, including a paracetamol overdose and an oxycodone overdose. So quite hard to unpit. Yeah, definitely. We didn't have any tizanidine alone cases.

No, they were all combined with other drugs, which does make interpreting the level and its significance quite challenging. One of the suicide cases had a concentration of two micrograms per litre, and it was queried that this might have been due to prolonged metabolism of tizanidine. So the individual had taken overdose was found brought to hospital and the sample that was analysed was taken four hours after their admission. So it's quite hard to know how that relates to the time of their overdose and what sort of concentration they did take.

As you said, it's quite short acting, isn't it? So relatively short half-life. So I do find tizanidine is a really interesting drug. We don't encounter it a huge amount, but it is very occasionally prescribed, isn't it, by specialist prescribers out of interest.

Do we know if of these cases where they all prescribed it? I don't know. I don't remember reading about information in the paper. It's probably not a typical drug of abuse.

But then I'm often surprised in this job. So you never know. Exactly. One key thing that I don't think they talked about in either the medetomidine paper or the tizanidine paper, post-mortem redistribution.

They did mention it in the tizanidine paper that it might have influenced the concentration seen because it's not known how tizanidine behaves during that post-mortem interval. Yeah, it's completely fair that interpreting anything post-mortem needs to be considered, doesn't it? What changes occur and does this concentration I'm measuring in my post-mortem blood sample actually reflect what happened at the time of death? So in addition to acting on the alpha-2 adrenergic receptor, tizanidine is also known to act on the imidazoline receptor, which has been shown to have antinociceptive activity, so it acts to relieve pain.

And I had to Google this receptor because I had never heard of it. It just shows how complicated the human body can be, actually. Potentially a whole other line of activation for not just this drug, but

possibly medetomidine and xylazine. I'd need to have a look and see whether they might all act through this pathway, too.

Let's focus a little bit again on xylazine and medetomidine. We talk about them as being contaminants in the drug supply. Is this language completely wrong? Should we be thinking of them as part of the drug?

Potentially. I mean, it's quite tricky when it's mixed in with the heroin or fentanyl. People are buying it with the intention of getting either heroin or fentanyl and not necessarily all of the other things that are mixed in. So in that sense, it kind of is, quote unquote, contaminating that sort of drug supply.

But I'm sure people might be seeking out these drugs as we've seen with things like nidazines. I think we've certainly been some reports, hasn't there, of people deliberately seeking fentanyl containing xylazine? Which I find crazy because a lot of the reports from users on Reddit really don't get on with xylazine, and it puts them off a whole host of different drugs because they're worried that they're going to end up with something contaminated with xylazine. But potentially, looking at that rat data, maybe there's something actually pharmacological going on with reward and relapse pathways.

True. Yeah, very interesting. Let's go back briefly to the medetomidine paper. You talked about enantiomers, not necessarily something anybody is routinely doing, even if they are doing medetomidine detection.

So again, is this another area where potentially there's a bit of a blind spot? We need to be considering enantiomer separation, particularly if we're going to measure medetomidine and try and interpret its concentration. We need to know whether it's purely dex or whether it's a mix, right? Yeah, and I think it also has its use of pointing to the source of it, whether it's been made or diverted veterinary meds, or whether it is being given in a sort of emergency department setting.

And that's why we're seeing it. Yeah, that would be really helpful information, wouldn't it, potentially. You're going to make me start googling chiral columns again and start thinking about this, aren't you? I mean, I think it's something we need to be thinking about.

So where should the research go next? It's really nice to see these two pure research papers looking at the effects of xylazine and trying to unpick some of what we might be seeing on a epidemiological level and in toxicology casework. But where's next? It's a really good question.

I'm not sure. Potentially looking at alpha-2 reversal agents, which perhaps might be beneficial in some emergency situations. So this question is possibly out there, but we've seen xylazine and we've seen medetomidine move into the opioid circles. Is tizanidine going to do the same thing?

Possibly. I think only time will tell with that one. I think unlike xylazine and medetomidine, there's not a big veterinary supply. As far as I'm aware, I could be wrong.

So possibly we're not going to see it in quite the same way. There's actually a whole host of other alpha-2 agonists out there. It's a class of drug I wasn't really very aware of until I came across xylazine and then started looking at other drugs like it, possibly a really underappreciated class of drug. So I do think we need to be thinking about their role, perhaps not directly contributing to toxicology, but perhaps having a synergistic effect.

As we've seen in pretty much all of these cases and indeed the experimental data, it wasn't an alpha-2 agonist on its own at any point, really. It was an alpha-2 agonist actually attenuating opioids and other drugs. And drug interactions are something I think we don't do particularly well at. And so I think we should really be considering them as we interpret some of this data.

Yeah, no, definitely. So one final question, then. What's the take home message? What are the implications of these papers for today?

I think these are definitely things that we need to be thinking about. And it's a class of drug that does need to be on a radar and having papers like the Tizanidine paper and the medetomidine paper to aid interpretation of sort of drug levels you might see is really important. Yeah, for sure. I think I've seen one case of Tizanidine post-mortem and I don't think there was anything at the time to compare it to.

So this is actually really, really helpful data for people like us. I think as well it really is a case of watch this space, isn't it? We know that maybe in the UK Zylizine isn't quite as prevalent as it is in the US. And possibly we've seen a little bit of medetomidine based on that paper, but certainly not a drug I've come across.

And yet it could be out there and we need to be aware of it and make sure we are looking for it when appropriate. OK, so I think we're going to wrap it up here this week. I do hope you've enjoyed listening. If you did enjoy, do maybe give us a like on socials or give us a five star rating on Spotify.

That would be really appreciated. Hope you all have an amazing week and we'll see you next time. Bye.