Episode 51: Brorphine - Could Orphines Be the Next Nitazenes?

Transcript

I'm Rebecca. I'm Rob. This week, we're going to be taking a look at brophine analogues, or orphines, as they're becoming increasingly known, novel synthetic opioids, similar to nitazenes. We're going to be looking at what they are, where they've come from, and whether we need to be worried.

But before we dive into that, Rebecca, how are you feeling after our birthday episode? I'm really, yeah, really pleased, and thank you to everyone who reached out and commented on our posts. We really appreciate it, as always, so thank you. So last week was the birthday episode.

This week, the hangover episode. Yes. No, no, we're going to be diving back into some raw science. So yeah, should we dive in?

Yeah. 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 page on LinkedIn, and we're really active on there. And for those of you who don't use social media, but still want to get in touch with comments about episodes or episode suggestions, you can email us at thetoxlab@gmail.com.

So brorphine analogues are this class of novel synthetic opioids, and they've got a really rich history. In 2018, brorphine was first described in a paper from a group claiming to have discovered a series of opioids which actually had a better safety profile. They were looking at what we call biased agonists. So when opioid drugs activate the mu opioid receptor, there are two complementary pathways that activate following this binding.

And one of them is the G-protein response, and the other is the beta-arrestin response. It's thought that the G-protein-coupled response is what leads to the painkilling potential, the analgesia. And the beta-arrestin response is what leads to the respiratory depression, constipation and tolerance. And so the hope was that they had discovered this series of opioids.

And hidden in that paper, they described number 21, which was a brominated version of this core structure they had synthesised. This was 2018. But we actually need to look back a little bit further in history. Paul Janssen, a famous Belgian pharmacologist and doctor, discovered a huge range of drugs.

Maybe we could do a whole episode actually dedicated to his career, but he famously invented, among other things, fentanyl. But in the 60s and 70s, his group were experimenting with what they called R-series opioids. And these were essentially the same core structure as the group who described brorphine in 2018. So these are the first two descriptions of this class of compound.

But it was in 2020 when brorphine started to appear on drug markets and in toxicology casework. And we think this is where the name came from: bro, or bromine, because it had a bromine group, and orphine because it acted at the mu opioid receptor like morphine. Structurally, it has nothing to do with morphine.

So Rebecca, why don't you take us through a case report where brorphine was detected? This first case involves a 24-year-old male who presented to the emergency department with withdrawal symptoms, seeking admission for detox. The complaints he was experiencing included generalised

pain, mainly in the chest and abdomen. He presented with a normal blood pressure and a tachycardia at 114 beats per minute.

His oxygen saturations were 98% and he had a temperature of 37.3 degrees C. He presented with confusion, generalized weakness and cramping, and he was clammy and sweaty on physical exam. They went on to do a CT of his brain, a chest X-ray and an ECG, which were all normal. The only abnormality on his bloods was a slightly raised gamma-GT.

The patient reported a history of opioid misuse, including the use of methyl-AP-237 and IVSL methyl ketobemidone. Let's talk about this in language we understand, right? What are these things? So methyl-AP-237 is an analogue of AP-237.

AP-237, also known as bucinnazine, is a synthetic opioid that was used quite a bit in China to treat cancer pain. So it was a legitimate pharmaceutical and he was buying an analogue version of that. As I said, methyl ketobemidone is an analogue of the drug ketobemidone, which is an opioid drug. I don't know how often it's used, but it was developed back in the 50s.

So he was regularly using unlicensed opioids sourced from a marketplace somewhere? He had had a year-long abstinence from drugs up to about six months before his presentation, but one to two months before presenting to hospital, he had started using brorphine orally four times a day, as well as etizolam, which is a benzodiazepine. He reported that brorphine resulted in an effect similar to oxycodone or fentanyl, with a high that lasted quite a long time, and he'd also developed tolerance. He described intense cravings which were more intense than heroin.

Alongside this, he was prescribed a variety of drugs, including sertraline, mirtazapine, methylphenidate and enoxaparin. So before he entered treatment, he presented to the emergency physician two powders that he'd obtained from a Dutch marketplace labelled brorphine and etizolam, so these were submitted for testing. And they also had access to two serum samples taken 60 hours apart to test for the presence of these drugs. One was from his admission, and the second one was during his hospitalisation in the psychiatry ward.

These samples and the powders were screened and quantitated using high-res MS, and they were also able to assess in vitro biological activity at the mu opioid receptor using a beta-arrestin recruitment assay, which we've talked about before when looking at nitazenes. Analysis of the powder using high-res MS confirmed the presence of brorphine, and this was also confirmed using GCMS. It appeared that no impurities were found in this powder. In the in vitro mu opioid receptor activation assay, the brorphine had a similar potency to hydromorphone, but was twice as efficacious at activating the mu opioid receptor.

Initial toxicology screening on the serum samples showed the presence of sertraline, mirtazapine and risperidone, which was given to him during his hospital visit. But in these samples, etizolam, acetaminophen, ketobemidone and methyl-AP-237 were not detected. Brorphine was confirmed in both serum samples and was present at a concentration of 69.4 nanograms per millilitre in the admission sample, and 7.9 nanograms per millilitre in the second sample. And they also managed to detect a brorphine hydroxy metabolite in the first sample.

They were also able to use the same opioid receptor activation assay, and showed there was opioid activity in the admission serum sample. So a man presents to hospital with clear signs of opioid withdrawal. He's got a history of using novel opioids, including what he believed was brorphine, went into hospital for treatment, inpatient detox. The powder and his blood all subsequently tested positive for brorphine.

And also, the activity assay showed that brorphine is a highly potent activator of the mu opioid receptor. And this was maybe one of the first case reports of brorphine, wasn't it? I believe so, yeah. It showed that it was present on marketplaces, and that it was a very potent opioid, perhaps not as potent as some of the nitazenes we've seen, but still much more potent than morphine, for example.

So at this point in time then, brorphine was sort of on toxicologist's radar, wasn't it? We knew it was available on marketplaces. There were isolated case reports like this one of people presenting to health care, and it occasionally turning up in post-mortem work. The CSFRE had identified it in a few fatal cases across the US and things like that.

So it was, I think at that point, thought this could be a concern. So as a result, there was a little bit more research that went on, wasn't there? And we found another paper looking at some of the metabolism and breakdown of brorphine. So this paper is looking at both in vitro and in vivo metabolism of brorphine using both pooled human liver microsomes assays and genuine blood and urine specimens from routine casework.

And they also looked at mu opioid receptor and kappa opioid receptor activation via a G-protein recruitment assay versus the beta-arrestin recruitment assay, which we talked about earlier. So in this paper, they had one urine sample from a 22-year-old male, femoral blood from a 25-year-old person, and they also had a serum sample, but there was no information attached to this case. They used a liquid-liquid extraction method and analysed these samples using LC-MS/MS.

For the in vitro metabolite investigation, they were using liver microsomes to generate phase one metabolites and then using a QTOF in both the in vitro models and the genuine samples to detect metabolites. And they were using criteria such as a mass error of less than five PPM, making sure the isotope pattern matched and that it had a signal-to-noise ratio greater than three to one. So in the urine sample, brorphine was present at a concentration of 38 nanograms per millilitre. In the femoral blood sample, brorphine was present at 1400 nanograms per millilitre.

And in serum, due to the lack of sample, they could only tell that it was greater than 20 nanograms per millilitre. 1400. That sounds quite high for a fairly potent opioid. It does.

So in the in vitro assay, six phase one metabolites were identified through oxidative N-dealkylation, monohydroxylation, N-oxide formation or monohydroxylation followed by a methylation. The monohydroxylation and N-oxide formation metabolites were the most prominent detected in the microsomes models. And you also see some stereoisomers of the monohydroxylation metabolites. So you can get hydroxylation in three different positions.

In vivo investigation saw 11 metabolites in urine formed by oxidative N-dealkylation, mono and dihydroxylation, N-oxide formation. And then you've got phase two sulfate conjugates as well. In blood, however, only N-dealkylation and the three stereoisomers from the monohydroxylation were confirmed and N-oxide was the most abundant in vivo metabolite. But this wasn't present in blood.

They did do some hydrolysis of the urine sample, which did increase the detection of two of them on a hydroxylation metabolites, which were presumed to be bound to glucuronic acid to aid excretion. This all works really, really valuable, isn't it? Firstly, it gives us extra targets to look for when we're looking for these compounds, because sometimes detection windows of potent novel drugs actually might be quite short. And so being able to look for metabolites can actually make it easier to spot what you're looking for.

Sometimes you can have a bit of a guess as to how variable metabolism might be between people and show whether there might be a risk of differences in toxicity between individuals due to differences in metabolism. And it does seem, based on the metabolite produced through N-dealkylation, that this

might be common to lots of different orphines. So it'd be a good general metabolite marker to look for. But obviously we've only got case data from one individual so we don't know how abundant this metabolite will be or where it will necessarily show up in all casework.

Yeah, you've just sort of teased the future a little bit, haven't you? We're talking about brorphine at the moment, but there might be a little bit more to that. So looking at receptor activation, they used a radiolabelled GTP-based receptor activation assay to basically determine how well brorphine activated both the mu opioid and kappa opioid receptors. And based on this assay, brorphine is more potent than hydromorphone and is a full agonist at the mu opioid receptor.

And then brorphine also appeared to be a partial agonist at the kappa opioid receptor, but it's far less potent compared to the two U-series reference compounds, which we know have activity at the kappa opioid receptor. So mostly these are mu activators, which is what we expected. And that fits the pattern that we saw in that patient who presented in withdrawal. And it is important to note when looking at the different types of receptor activation assays that depending on which one you're using, they do vary in the results.

They make it quite hard to compare between different recruitment assays. So if you are reading lots of papers and they do look to be a bit different, that's just something to be aware of. Yeah, you need to compare within the same assay conditions, don't you, to actually be able to try and unpick a lot of this? So, and we need to be clear, we're still in the early 2020s at this point of our timeline, aren't we?

Brorphine was here, scientists were doing research identifying metabolites, people were presenting to hospital. It was on toxicologists' radar, it was on the government's radar. And as a result, brorphine was banned. So brorphine was made Schedule I of the Controlled Substances Act in December 2020.

And this came into effect in March 2021. And the UN reported that brorphine was seen in four different countries in 2021. After the ban, it was only seen in one in 2023 and 2024. So why then in 2025 are we looking at the story of brorphine?

It came, it went, it's no longer here. Well, in the novel synthetic opioid world, things happened in 2021. We saw the rise of nitazenes. And nitazenes became prevalent globally and to an extent are still around today.

And nitazenes we've spent a lot of time talking about on this podcast, haven't we? They have been a big part of emerging toxicology for the last two years. But crucially, what has happened this year is that China has now enacted a generic ban on nitazene structures. So the key question is, are Orphine analogs going to make an appearance?

So the UN produced an update in May of this year and several brorphine analogs, including 5,6-dichloro-desmethyl-chlorphine and propionitrile-chlorphine, also known as cyclothine and Orphine were reported to them in 2024. And R6890, also known as spirochlorphine, were reported in 2025. And both cyclothine and spirochlorphine have been reported in both Europe and North America. So we're possibly now entering a post-nitazene era whereby Orphine analogs are now the next thing on the radar.

So there has been a little bit of in vitro characterization of some of these orphine analogues by Marta van der Pijl, who's indicated that chlorphine, fluorphine and iodorphine were generally the most active mu opioid receptor agonists. And in mouse experiments, chlorphine and brorphine induced the highest level of antinociception and different analogues produced pronounced respiratory depressant effects with observed differences in sensitivity to naloxone. So the effects that we're going to see are going to be a little bit variable depending on the orphine analogue that we're dealing with.

And that makes everything just that much trickier, doesn't it? It means users are going to find dosing harder because they won't necessarily know what Orphines they're dealing with. As toxicologists, it makes interpretation harder. So where does this all leave us?

Are we staring into the abyss of a new novel opioid epidemic? Possibly. There are a lot of Orphine analogs, aren't there? Oh, yes.

They've been described. The Janssen work describes quite a few of these R series. And some of them do cross over. So cyclothine was also in an R series paper back in the 70s.

So make sure your libraries are up to date. That was our job this week, wasn't it? Oh, yeah. That was an uphill struggle.

That was a task. It was. Of course, we don't actually know which of these analogues, if any, are going to emerge. But I think there is generally now a concern that they could in light of the generic ban in China on nitazene analogues.

And some of these are clearly quite potent and possibly unpredictable. So they are definitely things that we need to be aware of and thinking about. Yeah, and it's definitely a challenge for toxicologists to try and keep libraries up to date so that we can be ahead of the curve with detecting Orphines or whatever drugs around the corner. The other challenge for some of these is that they've got a lot of potential halogenation sites.

And so swapping out a chlorine for a bromine or an iodine is a fairly common strategy to generate new molecules that are structurally very similar but different enough to trick the mass spec and the law. And so given they've got so many halogenation sites, it does make you wonder how many possible variants might actually start to emerge. So really in summary then, brorphine was here and is no longer here because we saw the rise in the nitazenes. But now nitazenes are possibly fading away.

Orphine analogs are gonna be perhaps in the spotlight. They're potent, they're complicated. There's a lot of potential variations and analogs that could be made, that could emerge. And it's possible that they're gonna be appearing in our work very, very soon.

So I hope you've enjoyed listening to us this week. If you have enjoyed it, do give us a like, give us a follow, we'd really appreciate that. Hope you will have an amazing week and we'll see you next time, bye.