THE TOX LAB

Podcast Transcript

Episode 14: Have we reached peak Nitazene?

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TRANSCRIPT

Welcome back to The Tox Lab. I'm Rebecca.

I'm Rob. This week we're going to be having another look at nitazenes.

We first looked at nitazenes a few weeks ago, and this week we're going to be looking at the structure-activity relationships of nitazenes and how different nitazenes vary in their characteristics.

Before we get into that, I want to briefly go back over what nitazenes are and why we care about them. Nitazenes are novel opioid drugs. They were first developed in the 1950s by a Swiss pharmaceutical company that was looking for alternatives to existing opioid drugs for medical use, and they synthesised etonitazene.

They tested it, patented it, and ultimately decided it showed no clinical benefit and had significant adverse effects. It was incredibly potent and caused marked respiratory depression, so nitazenes were largely forgotten about.

Then, in 2019, there were the first reports of nitazenes emerging as drugs of misuse, and since then they have slowly started to appear in forensic casework, post-mortem cases, and drug seizures. Very soon we then had this whole wave of nitazene analogues, or nitazene variants.

Today, we often encounter nitazenes mixed with heroin, in counterfeit medications, and sometimes sold as powders in their own right, and they really have turned parts of toxicology upside down over the last couple of years.

So Rebecca, with that in mind, why don't you take us through the paper we're looking at today?

As Rob mentioned, nitazenes are novel synthetic opioids. Opioids are a class of drug that act primarily at the mu-opioid receptor in the brain, and their main role is to provide analgesia, or pain relief.

Other drugs in this class include morphine, codeine, and fentanyl, all things most people will have heard of. They can also produce sedation, euphoria, and nausea, and at higher doses they can cause respiratory depression, which can be fatal.

The opioid system is also part of the body's own way of modulating pain. If you hurt your foot, for example, it hurts immediately, but after a while that pain fades a bit, and endogenous opioid signalling is part of that process.

Opioid drugs basically hijack those same receptors. Medically, they are very useful because they can reduce pain, but if you stimulate those receptors too strongly, they can also cause euphoria and, ultimately, fatal respiratory depression.

Nitazenes, or 2-benzylbenzimidazole opioids, which is a bit of a mouthful, are a broad structural class of synthetic opioids. You can modify that core structure in different ways, and those changes alter how strongly the molecules bind to the mu-opioid receptor and how potent they are.

This study looked at a set of 15 different nitazenes, including some that had been predicted to emerge but had not yet been seen in the drug market. The researchers looked at substitutions at three positions on the core structure that were already known to affect potency, and one previously uncharacterised position. They then used a range of in vitro, cell-based assays and some in vivo mouse experiments to see how those changes affected receptor activity and potency.

So they took a core nitazene structure, tried lots of different chemical variations at different positions, and then looked at how well each analogue interacted with the opioid receptor.

Exactly. One way to think about it is that the receptor is a lock and the drug is a key. The question was: which of these modified molecules produces the best fit, and therefore the strongest effect?

The first experiment they carried out was a radioligand binding assay. This was designed to assess how strongly these nitazenes bind to the receptor, in other words their affinity.

For this they used a radiolabelled synthetic opioid peptide called DAMGO, which binds to the mu-opioid receptor. They then introduced the nitazenes and asked whether those nitazenes would displace DAMGO from the receptor. The more strongly a nitazene binds, the better it is at competing DAMGO off the receptor.

So it's a bit like musical chairs. The receptor sites are the chairs, DAMGO is already sitting in some of them, and then the nitazenes come in and try to push DAMGO out. If the nitazene binds really well, it wins the seat.

Exactly. What they found was that all 15 nitazenes bound with nanomolar affinity, so they were all clearly active to some degree. One of the compounds, 5-methyl-N-desethyl isotonitazene, had the highest affinity of the 15, while ethyleneoxynitazene had the lowest affinity.

So all of these analogues were active, but some bound more strongly than others.

Yes. And 5-methyl-N-desethyl isotonitazene actually had higher affinity than some nitazenes we already know about, whereas others had lower affinity relative to reference compounds like fentanyl and morphine.

Once they had assessed how well these compounds bound to the receptor, they then wanted to know whether they actually activated it, because binding alone does not necessarily mean receptor activation.

The mu-opioid receptor is a G-protein-coupled receptor, and when a ligand binds, the receptor changes shape and can recruit signalling proteins. In this study, they used a beta-arrestin recruitment assay.

They took a luminescent enzyme called NanoLuc and split it into two halves. One half was attached to the mu-opioid receptor and the other half to beta-arrestin, which gets recruited when the receptor is activated. When a ligand binds and activates the receptor, beta-arrestin is recruited, the two halves come close together again, and when substrate is added you get a measurable bioluminescent signal.

So in the first assay they were asking how well these opioids bind to the receptor, and in the second they were asking how well they activate it.

Exactly. This is done across a range of concentrations so you can generate a dose-response curve. That gives you information about potency, meaning how much of the drug is needed to produce a given effect, and efficacy, meaning the maximum effect the drug can produce.

So a drug can be very potent, meaning it produces an effect at a low concentration, and it can also have high efficacy, meaning it activates the receptor very strongly once it gets there.

In this assay, etonitazene was the most potent of the characterised nitazenes and ethyleneoxynitazene was the least potent of the 15 they tested. Interestingly, methylenedioxynitazene and alpha-methyl etonitazene were the only new nitazenes in this study that showed greater potency than fentanyl.

So although all of these modified nitazenes were active to some extent, many of them were not actually more potent than fentanyl in this particular assay.

Yes, and interestingly, some of the modified butonitazene analogues they looked at, such as isobutonitazene and sec-butonitazene, were not more potent or more efficacious than the parent compounds. So those modifications did not enhance activity.

After the in vitro experiments, the researchers then selected six nitazene analogues for in vivo experiments in mice to see how they behaved in a whole-animal model.

They looked at protonitazene and five potent analogues of etonitazene, including ethyleneoxynitazene, 5-methyl-N-desethyl isotonitazene, 1-ethylpyrrolidino etonitazene, alpha-methyl etonitazene, and etylene etonitazene.

These names are absurdly long.

They really are. The researchers looked at three main features: locomotor activity, body temperature, and hot plate latency, which is basically how long it takes a mouse to respond to a painful heat stimulus.

Biologically, that hot plate test is really a marker of analgesia, isn't it? You are effectively measuring how much the pain response has been suppressed.

Exactly. All six nitazenes showed dose-dependent effects in these categories. They all reached maximal levels of blocking the painful stimulus. The most potent in the hot plate test was alpha-methyl etonitazene and the least potent was ethyleneoxynitazene.

Interestingly, as the dose increased, there was a fairly linear decrease in body temperature and an increase in antinociception, meaning pain suppression.

For locomotor activity, they saw a biphasic response. At lower doses, movement decreased. At intermediate doses, the distance travelled increased, and then at higher doses it decreased again.

So there seemed to be some kind of mid-dose stimulatory or excitatory effect going on?

Yes, that is probably the simplest way of putting it.

These 15 nitazenes were, at the start of the study, compounds that had not yet been seen in the drug market. They were predicted analogues based on what we already know about nitazene structures and the kinds of substitutions people make.

But by the time the study was completed, four of them had already appeared on the recreational drug market. Those included 5-methyl-N-desethyl isotonitazene, ethyleneoxynitazene, etylene etonitazene, and isobutonitazene.

So they successfully predicted the emergence of some of these compounds.

Yes, and it will be interesting to see whether more of them appear, or whether some do not emerge because they simply are not potent enough to be attractive to whoever is making or supplying them.

So is there anything we can learn about the structure-activity relationships of nitazenes from this study?

Yes. Broadly, we already knew that removing certain groups, such as the nitro group, tends to reduce potency. The study also suggests that chain length matters, but not in a simple linear way.

There seems to be a kind of sweet spot. Relatively short chain lengths may be more potent than either very short or longer linear chain lengths, and some substitutions with bulkier or different functional groups can clearly reduce activity.

So in the wider context of nitazenes, we have this large collection of chemicals. They are all slightly different, and they all have different potency characteristics. Some are extraordinarily potent, while others are less so, although even the less potent ones are still very potent compared with more traditional opioids.

Yes. Even the least potent of these compounds is still potent in the wider opioid context.

How did the receptor binding and beta-arrestin data compare with the mouse potencies?

They only took six of the more potent compounds through to the mouse experiments, so we do not know whether some of the weaker-looking compounds in cell-based assays might have behaved differently in vivo. But that raises an important point: when you alter a chemical, you are doing more than just changing how well it fits the receptor.

Exactly. You are also affecting things like bioavailability and how well the drug gets into the brain. A compound might fit the receptor slightly less well but still have greater overall effect in vivo if it crosses biological barriers more effectively.

Nitazenes are quite lipophilic in general, so they are already fairly bioavailable compounds. But it would still be interesting to know how well these animal findings translate to humans.

Completely. We also know from other work that metabolites can complicate the picture. For example, there is another study looking at isotonitazene and N-desethyl isotonitazene. N-desethyl isotonitazene can show higher receptor activity in vitro, but in a mouse model it appears less potent overall, probably because it is more polar and less able to get into the brain effectively.

So there is still a lot to learn, not just about parent nitazenes but also about their metabolites, and whether some of those metabolites may themselves be active or even appear as drugs in their own right.

That is a really good point. The in vitro assays tell you something about one molecule at one receptor, but the whole-animal model introduces many more variables, including metabolism, tissue distribution, and bioavailability.

And as some metabolites, like N-desethyl protonitazene and N-desethyl isotonitazene, have appeared as drugs in their own right, it becomes even harder to interpret toxicology results. Are you looking at a metabolite from another nitazene, or are you looking at direct use of that compound?

It is still such a raw, developing area of toxicology, and everybody is trying to make sense of it in real time.

I do have to give a big shout-out to the team who did this work. Nitazenes have taken over our lives for the last couple of years, and without this sort of fundamental activity data we would have very little to go on when trying to interpret what any of these compounds mean in the real world.

So I guess the big question is this: all of the nitazenes studied here, although some have now emerged in the drug market, were less potent than some of the earlier nitazenes we already know about. In other words, this study did not identify a brand new nitazene that was suddenly far more potent than etonitazene or N-desethyl isotonitazene.

So what is next? Will nitazenes continue to evolve but gradually become less potent? Have we reached peak nitazene?

That is a very good question. I do not think anyone really knows.

I hope they do not get any more potent, because as things stand they are already terrifyingly dangerous and doing a huge amount of harm.

I was actually thinking about carfentanil in that context. Carfentanil is a fentanyl analogue that is around a hundred times more potent than fentanyl and is used to sedate very large animals. It is the kind of compound that makes you realise someone can always potentially discover something even more potent than what we already thought was extreme.

So the question is: is there a carfentanil version of nitazenes just around the corner that we have not yet identified?

I really, really hope not, because I cannot begin to imagine the impact if something even more potent than the nitazenes we already have made its way into the drug supply.

We have already seen nitazenes in heroin, but also contaminating things like counterfeit benzodiazepines, which is deeply concerning. The idea of something even more potent is awful.

So in summary: a number of novel nitazenes have been synthesised and tested in various ways, and although they are all potent opioids, the chemical modifications studied here generally seem to reduce potency rather than increase it.

These compounds have now been characterised, which helps us analytically because we know more about what to look for, but crucially the study did not uncover a dramatically more potent nitazene than the ones already known.

So perhaps we are at least past peak nitazene potency. But even if that is true, we may still continue to see new nitazenes appear, and that alone makes the area analytically and clinically very challenging.

I think we are going to wrap it up there. I hope you have enjoyed listening this week.

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Hope you all have an amazing week, and we will see you next time. Bye.