Episode 37: Nitazene Metabolites in Post-Mortem Casework

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Transcript

Hi everyone, welcome back to the Tox Lab. I'm Rebecca. I'm Rob. This week we're going to do something a little bit different.

I'm often told, I don't know if you get this Rebecca as well, having your own science podcast, quite self-indulgent. Well we're going to take it to the next level and we're going to talk about some of our own science on the science podcast. I'm very excited. So we're going to be looking again at the subject of nitazenes, novel opioid drugs that have been emerging in the drug markets over the last five or six years but much more significantly in the last two or three or so.

And we're going to be looking at how they break down, what that means and why possibly we should care. Before we jump into today's episode, if you want to stay up to date with our content and contact with future episode suggestions, you can find us on Instagram at @the_tox_lab. We also have a page on LinkedIn. And for those of you who don't use social media, you can email us at thetoxlab@gmail.com.

So Rebecca, our first paper this week, rather excitingly. I'm very proud of this. Why don't you introduce us to our first paper? So the first paper we're looking at today is a piece of work that we very recently published in the Journal of Analytical Toxicology, looking at different metabolites of protonitazine.

So protonitazine is one of the nitazene drugs. So it's a very, very potent novel opioid, two or three hundred times as potent as morphine, estimated. About that. Thereabouts.

So very, very significant. And it was an opioid drug that had been emerging in clinical work, in post-mortem work across the UK. And there wasn't a huge amount published on its breakdown. There was a couple of papers that had done liver microsome models.

So they had taken a cell line of liver cells, incubated it with protonitazine and looked for the breakdown products which were formed. But there was very limited real world data, wasn't there? There was previously published a single or a pair of cases in which some metabolites were looked at in urine. And so we had a fairly large data set.

We had seen protonitazine in a number of cases. And we thought, let's apply the same rationale and look at our data to see how many metabolites were present in that data set. We talked a little while ago about Hirer's accurate mass spectrometry and how that acquires a lot of data in the background that we don't normally look at. And so this was just retrospectively mining cases we had already analysed.

And so we looked for N-desethylprotonitazine, 5-aminoprotonitazine, 4-hydroxynitazine, N-desethylhydroxynitazine and hydroxyprotonitazine. A lot of very long words in that list. It is. So in other words, we figured out what the masses for these metabolites should be.

We knew what their fragmentation pattern should be based on either previously published data or predicted it and then dove into the data to see which metabolites were present in each case. So across these different cases we had a mixture of blood and urine and we'd analysed them using our very

general extraction aimed at extracting neutral and basic drugs, and then we also developed an optimised extraction for nitazenes. This was something we had done a little while previously, hadn't we? We were aware that our regular extraction method was just not clean enough.

We were not getting the limits of detection we needed for these very potent drugs. And so we were using effectively a two extraction system. So across our blood samples with our routine extraction we were mainly seeing 5-aminoprotonitazine and N-desethylprotonitazine and in our optimised extraction we were seeing both of those but we were also seeing hydroxyprotonitazine which at the time and still today as far as I'm aware has never been reported in real casework. It's only been seen in liver microsome models.

So the liver microsome models has seen this so it possibly is a metabolite. In our urine samples we were seeing N-desethylprotonitazine and 5-aminoprotonitazine but we were also seeing hydroxymetabolite so 4-hydroxynitazine and N-desethylhydroxynitazine and when using our optimised extraction again we were seeing 5-aminoprotonitazine, N-desethylprotonitazine 4-hydroxynitazine and hydroxyprotonitazine. So a range of different metabolites across the range of samples, different matrices, different extraction procedures. Were there any patterns?

So it seemed that the 5-amino and the desethylmetabolites were seen most often across both blood and urine and both extraction methods. The hydroxyprotonitazine that hadn't been reported previously was seen in the majority of cases in both blood and urine on that optimised extraction. Whereas with the 4-hydroxynitabolite and the desethylhydroxynitabolite you were only really seeing them in urine I think we saw them in one case in blood but mainly they were detectable in urine. This is consistent with more polar or more processed metabolites occurring in the urine.

And it is important to mention that routinely we don't hydrolyze our urine samples which a lot of other labs do so it might be that we would see other metabolites in the urine that we weren't able to detect because of the way our work was done. Like this was all a retrospective data analysis so no extra analytical work was done. Yeah that's completely fair. Are there then glucuronide metabolites being formed later on that we simply wouldn't have seen in this case?

I guess the important question is why do we care? Well there was one paper that came out a little while ago that looked at metabolites of protonitazine and said that we should be screening for 4-hydroxynitazine because that's the most common metabolite you detect and you see quite nice peaks for it across different samples. This was based on a 2-sample analysis. Yeah, whereas in our data we've shown that you don't really see the 4-hydroxy in blood you see it most often in urine and even then I think it was only present in about half our cases.

In the metabolism or the metabolites we see across all these different cases seem to be so variable we couldn't see a pattern bearing in mind our data set is still quite small so it seems that you can't get away with just screening for one or two metabolites you want to be looking for as many as possible to increase your ability to detect protonitazine exposure. Yeah I think that was really the key finding here wasn't it?

That there wasn't an obvious relationship between the signal based entirely this is entirely crude based on peak area the signal of protonitazine and any of the metabolites there was so much variability some cases there was more of one metabolite some cases there was more of another potentially a few factors differences in stability potentially differences in individual metabolism So I think it was in the liver microsome models they were looking at the sort of enzymes that produce these metabolites you've got enzymes like CYP2D6 CYP2B6 and CYP2C8 which are known to be a highly polymorphic so could potentially account for the variability we're seeing across cases but there are so many different factors you've also got to account for potential post-mortem redistribution we've

talked about this before we don't know how nittyscenes behave in that post-mortem interval we have some idea that they're possibly not behaving in a way that is nice so depending on how long that interval is that could potentially account for why you're seeing some and not others because of just the way they're redistributing and then layer on to that obviously post-mortem redistribution of the metabolites themselves could well behave in very very different ways almost certainly will given that some are much more polar than others I think that takes us on to a key point doesn't it if there is a lot of genetic variability for how these nittyzines are metabolised and crucially some of these metabolites are active aren't they? yes so we know that ndazethal protanitosine and 4-hydroxynitosine are active although less so ndazethal protanitosine about 3-6 times less potent than protanitosine and the 4-hydroxys between 80 and 160 times less potent than protanitosine so whilst still active they are less potent but the other ones so the dazethal hydroxy the 5-amino and hydroxyprotanitosine there is no potency data out there as of yet and one of these could potentially be slightly more active than the other ones and could therefore potentially contribute towards toxicity we also don't know how well they cross the blood-brain barrier for example and therefore how well they are going to act even if they do reach the receptor yeah that's really fair isn't it that's another quirk of some of the nittyzine metabolism we saw this with isosynitosine and ndazethal isetanitosine didn't we whereby ndazethal isetanitosine in cell models actually had higher potency at the receptor but in animal models that wasn't quite the case and we think it was to do with it was more polar so it wasn't quite crossing into the blood-brain barrier in quite the same way as the parent drug was so this does all complicate interpretation of potential toxicity doesn't it if you've got a drug behaving differently in different people potentially you might see different results from a similar dose yeah it is quite messy and another thing we've seen it with ndazethal isetanitosine ndazethal protanitosine is emerging as a drug in its own right as well as a metabolite of protanitosine so when you see it you can't always be sure whether it's there because it's as metabolite of protanitosine or whether it's also there because it's mixed into the drug supply and that also complicates interpretation further yeah that's another good point as well actually and the amino as well 5-amino isetanitosine emerged as a drug in its own right I'm not aware of 5-amino protanitosine yet but there's no reason it couldn't very true so there are some important caveats with our data set one, for a lot of these the 5-amino protanitosine the dazethal hydroxynitosine and the hydroxyprotenitosine there are no reference standards available so when we're saying they're present it's purely based on accurate mass based on the pattern of fragments we were expecting based on how we know ndazethal protanitosine is fragment in our system so they're not a guarantee another important point is that some of these could be present as degradation products within the sample rather than metabolites yeah I think that's a really fair point actually we know because we went fishing for these off a basis of microsome models there is reason to think they will be produced as metabolites but are they also as a result of either chemical instability or bacterial degradation entirely possible now this actually leads us on really really nicely to our next paper which we're going to look at today Rebecca introduces us to this paper so this next paper is looking at the detection of 5-amino metonitazene and 5-acetamido metonitazene in a post-mortem case so in June 2023 this lab received samples that were collected at post-mortem for analysis they received 1 preserved blood sample 2 un-preserved blood samples and 1 preserved urine sample and these were screened using LC triple quad, MS and they first tested the un-preserved sample and detected metonitazene at a concentration of 1 microgram per liter so really really tiny they then wanted to use their LC-QTOF to re-quantitate this in the sample and there was a delay of about 86 days between first analysis and the second analysis and when they tried to look for it on their LC-QTOF they couldn't see it it was below their limit of detection they then went and retested all of their samples on their triple quad and they could see metonitazene in the preserved blood sample in the preserved urine sample but it wasn't present in either of the un-preserved blood samples so they were hypothesizing that metonitazene was being converted into something in vitro in these un-preserved

samples so it was present in an un-preserved sample a couple of months later, 3 months later no longer present in the un-preserved sample, retested the other samples that were in the preserved blood sample present in both of those suggestion, therefore, is it's gone somewhere in the un-preserved blood sample so what they did next I find really interesting so nitrobenzodiazepines like clonazepam can produce an amino metabolite and then this can be further acetylated to produce an acetamidomatabolite but you can also get formation of this amino metabolite through bacterial degradation and this is highly variable depending on the type of bacteria present in the sample the storage temperature where there's preservative present and the pH and so they used this logic to look for both the 5-amino methanitosine metabolite and the acetamidomatin methanitosine metabolites and they did this using their LCQTOF and did find both the 5-amino and the 5-acetamido and the 5-acetamido methanitosine had a peak area about 30-fold larger than the 5-amino so it does suggest there was some conversion the fact that the methanitosine disappeared and then there was the detection of the amino and the acetamido hypothesis being methanitosine has become 5-amino and that further converted to acetamido and because these were un-preserved samples you've not got the preservative that's going to stop this from happening because they were selected at PM, you're potentially going to introduce some bacteria into your containers and that's not uncommon so this all kind of supports this theory.

They did also see a possible peak for 5-amino and azethyle methanitosine and 5-acetamido and azethyle methanitosine but they were very trace amounts and they didn't quite have enough fragments to feel confident in their ID but again reference materials these don't exist so they are like provisional conformations and 5-amino we were looking at protanitosine not methanitosine but we were seeing 5-amino as a metabolite we think so it's possible as we were saying looking at the first paper that there could be multiple pathways to formation of these products.

Some of these could be metabolites and some of them could also be bacterial degradation products. Yeah it is really hard to unpick for all of our cases all of our blood samples were preserved so that kind of limits the possibility a little bit of the 5-amino we were seeing being 3-bacterial degradation being present as a true metabolite. It might make it less like that it's bacterial degradation. Based on this paper we did go looking for 5-acetamido protanitosine and we only detected it in one of our cases and it was in a preserved blood sample and an unpreserved urine sample.

So it was only present in one and that sample was preserved. More recently because we know these potentially exist as markers for methanitosine and n-desethal methanitosine exposure they have been in the back of our minds and in a clinical case actually we detected 5-acetamido n-desethal methanitosine in an unpreserved urine sample but from selection to analysis that was all completed in about 3 days and it was stored at 4 degrees. Let's just break this down for a moment because you have just said an absolute mouthful there. Sorry.

Des-ethal so it was the des-ethal metabolite but it was the des-ethal acetamido metabolite of methanitosine. Yep. So it's undergone 3 biotransformations at that point. Yes.

And this finding really did highlight the complexity didn't it? Because I fully agree with the conclusions of the paper about how this looks like degradation bacterial degradation in a unpreserved blood sample. The fact that we detected an acetamido metabolite in a clinical sample 3 days after collection possibly indicates there might be more going on in terms of whether it could be a genuine metabolite as well. So lots of long names looking at lots of different metabolites and we've touched briefly on why we might care.

I think there's still a lot we don't know isn't there? Yeah it's such a messy space because these could be present as tumor metabolites, they could be present as products of bacterial degradation but I think

the important thing to remember is that these are only going to be present if a nitrogen has been there. So they are really useful as markers of exposure and increase your ability to pick these up. What it really means though in terms of interpretation, who really knows but I think they have their use.

Yeah I think that's really fair. I think the logical next step for any of this now is now we've identified these metabolites and we know they're present. The question is, going back to our paper whereby it was a retrospective analysis of all those cases that were positive for protonitazine, how many cases are there out there where there are metabolites but no protonitazine because of course we would never have picked that up with our retrospective study. And we have been screening for metabolites since then haven't we?

And I think we've had one or two cases where we've detected nitrogen metabolites but no parent drug. So there is real merit in looking for the breakdown products and as you say, whether they're true metabolites or whether they're breakdown products due to bacterial contamination or most likely a mixture of both it still tells you something about nitazine use it's such a complex area isn't it?

And it's complex because we go looking actually lots of drugs probably have got really really extensive metabolism pathways producing 5, 6, 8 metabolites most of the time we don't look we don't care but because these are new compounds because we don't quite know how they behave we're obviously invested a bit of time and resource in looking for these so I guess what's the take home message as always a question?

For me it's that we need to be screening for as many metabolites slash breakdown products as possible I think to help us pick up as many nitazine positive cases as we can particularly given the public health drive to try and identify these cases I would agree with you I think you probably do risk generating lots of data that is difficult to interpret but unless we do that we're not going to move anywhere are we? That's always the case it's very true and if it does turn out that one of these metabolites where we currently have no potency data for turns out to be quite potent and possibly important in interpretation then at least we have that.

Yeah that's fair for sure. Well I hope you've enjoyed listening this week, do check out the papers. Yep I'll link those in the episode description below as always and if anyone has seen any weird nitazine metabolite behaviour and wants to get in touch do so we'd love to showcase some more research like that on this podcast hope you all have an amazing week and we'll see you next time. Bye!