NMR spectroscopy for Structural Biology
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Lecture: 50
NMR in Drug metabolism III

Good morning students. So, this week we are discussing how we can use NMR in drug discovery and then like in last lecture I was discussing how we can use NMR in understanding the drug metabolism. So we discussed that drug metabolites passes through various body fluids. We can take those body fluids, and in vitro detect the level of drug and drug metabolites. Some of the obvious body fluids are urine, the plasma, serum, sweat, it can be spit, many of these body fluids can be used for understanding the drug metabolism.

The two famous one are plasma and serum and also urine that can be drawn. So NMR can give you quantitative assessment of drug metabolites. Also we looked at what are the nuclei that can be used for detecting drug metabolism. The famous one can be proton, and it can be carbon, then the best one appears to be fluorine in terms of sensitivity, in terms of the dispersion and the relaxation time. The problem with proton was like it is overcrowded by the intrinsic environment that comes in body fluids like urine.

So, getting the clear signal from drug which is in very low concentration can be often difficult. So, you need to combine with a 2D experiment when you can transfer the polarization from proton to X nuclei and detect on X nuclei to have a more clear understanding of drug metabolites. But those were all in vitro experiment. Can we also think of detecting the drug metabolites in in vivo system? So what is the good part of in vivo system that like one can measure the drug plasma level, but that does not always reflect the drug concentration at the receptor site. So plasma is circulating, it is in circulation, how much drug is circulating.

That is what we are measuring when we measure the plasma and drug concentration. But what happens the effective concentration that is reaching to receptor site that will not be directly reported once we measure the drug from the plasma. We need to have a method that can allow us to measure the concentration of a drug and their metabolites in situ and that will be very useful. We can measure wherever the drug should go and what is the concentration of that drug. If we can measure that, that will be wonderful.

So these experiments can be done if we actually wish to do in vivo study of drug metabolism. So the good part of the in vivo study of drug metabolism is that measurement of drug and metabolites can be done in the target organ, wherever drug is going can be directly measured from there. And another good part, that it can be repeatedly done on the same patient. So reproducibility or the variation that will come from batch to batch or measurement to measurement can also be accounted for and this is non-invasive, you are not taking anything out of just using some probe which is NMR based probe in noninvasive manner to detect the drug concentration at the site of its action. So that is the good part of in vivo study of drug metabolism. Let us go ahead and look at how we can do that and what we can do it.

Some drawback of this in vivo drug metabolism that generally it is very insensitive. It's sensitivity is 1 to 10 times less than in vitro concentration. Another big problem is like since these drug metabolites are not freely tumbling in the solution, so there is a unrestricted mobility because of surroundings. It can be coming from different proteins, lipids, muscle whatever it is. Since the mobility is restricted so you can understand that lines are going to be broad.

So if in vitro we have a sharp line, in vivo we can have broad line. That is one of the limitation of doing experiment in vivo because of restricted tumbling or restricted mobility. Now, because of sensitivity is low and also the lines are broader, you require longer data acquisition time, so many scans you have to do. Now, another problem can be that signal can come from various anatomical origins. It will not only come from the drug. In some cases like a fluorine which is a unique nuclei that is not found in the body, if your drug is fluorine label then you can get a signal exclusively coming from fluorine, otherwise if you are detecting say proton or carbon-13, it is obvious that signal will come from different anatomical origin molecules. Another problem will be resolution, it will be poor, so chemicals, chemically similar compound will be difficult to distinguish.

So the good part is in vivo detection of F19 and that can be actually detected and it has been done extensively of 5-fluorouracil which is a cancer drug and its metabolite has been estimated around 0.05 mM per gram that is what minimal you can detect from in vivo experiment. So some of these nuclei are really beautiful for detecting the signal of drug or drug metabolites in in vivo setting. So that is the application we can think of F19 based and we can look at what else we can do. So let us start with a F19 based nuclei.

So just to remind you why an F19, if you go few slides back in the last lecture we had just given you the idea about F19. I will just repeat few of those. So F19, the first good part of this is it is half nuclear, it's a spin angular momentum is half. It has relatively narrow line, sensitivity is very good, like about 83 percent of proton, it is a 100 percent natural abundance and the most important part that it has a short relaxation time T1 that means you can repeat this experiment very fast and the spectral width is very large. So you can distinguish the signal coming from different origin of F19.

So because of all these good part that F19 has, it can be used beautifully and exclusively in in vivo setting. So F19 study were used in anesthetic and psychoactive or anti-neoplastic drugs and like what these drugs has the F19 nuclei and it was used for detecting in vivo. So the distribution of anesthetic in the brain can be studied or even the pharmacokinetic of their elimination are studied. How it is being done is still a subject of controversy, but it can be used like you can detect it where it is going and how the pharmacokinetic is playing role. So the feasibility of in vivo F19 NMR in human has been demonstrated. One of the good example of this drug called fluoxetine, which is widely used for antidepressant drug.

This F19 drug was used and then you can see that this is the signal coming from it, just to give you an perspective. So once you administer this drug in the in vivo setting it can make some adduct or it can do some chemistry to make some other compound, it will also get metabolized, it can mix up with something. So this drug called fluoxetine actually make an product called norfluoxetine and these two are present in in vivo setting. So you can see that there are two peaks coming in the in vivo setting, coming around say 61-62 ppm, one is from norfluoxetine and another is from fluoxetine. If you detect the same thing in vivo you get actually the two peaks and these are the two peaks reflected. So first it gives confidence that we can get the clear cut two peaks. If you look at these are little bit shifted than this.

So the chemical shift can be different because you can see that the environment will be different and that is how chemical shifts are different. But good part is that since there are only two molecules which has a fluorine, we can conclusively, confidently detect this drug in in vivo setting. If we have done that then what next we can do? So we can do NMR study on this fluorinated drug like suppose 5-fluorouracil. So for 5-fluorouracil, F19 can be used because of various reasons. So first high dose administration can be done of these compounds and fluorine atom remains intact.

So fluorine is not mixing or it is not changing even during the biotransformation, even during the metabolism, F19 remains intact and that wherever they go in the metabolic cycle, you can detect it, take the signal coming from the various body fluid and you can detect it or even in in vivo setting you can do that. So fluorine atom remains intact during biotransformation, and F19 signal can therefore be displayed over a large spectral width without any overlap, and one can detect it. So you can see here we are also getting the fluoro-uracil and fluoride ion but some other product like a fluoro-melanoic acid semi-aldehyde or fluoroacetyldehyde. So these are mixing up and making some kind of another secondary metabolites but still the signal for fluorine remains intact.

So you can get an idea, where the fluorine is going, what kind of reaction it is doing and then what kind of product it is making and one can detect it. So, actually one can even do the fluorine-19 spectrum for the perforated body weight. So, you can take the F19. Here again the 5-fluorouracil, the fluoride ion and various other products that are coming. So this experiment was done, the drug was dosed to a rat, and then his liver was isolated and perfused, and it was found that the various catabolites that are coming from the rat liver. The catabolites of fluoro-uracil is not a fluoro-β-alanine, but metabolism continue to lead to new catabolites like a fluoroacetate or fluoro-melanoic acid semi-alcohol.

So these are metabolites that are coming up. So what we are learn here? We were injecting this 5-fluorouracil and then it is going in the body, doing reactions, and making new products. So that we are detecting new products from say rat liver. We are trying to prove the chemistry that goes on when these drugs are administered, what new products are made. So all these products we can essentially detect.

So you can see here is the concentration of FU, here is a fluoridine and whatever various products that it made, one can detect it and understand what new catabolites are made like a fluoroacetate or fluoro-melanoic acid semi-alcohol. These all products are made. So essentially, in vivo fluorine 19 NMR has been used to monitor the FU metabolism in the liver and it can be also used for understanding the metastasis of colorectal cancer in patients. So what you have to do? Like patients were treated with a continuous low dose of intravenous infusion of FU until the point of refractory of the disease and then you are doing something like doing the localized MRS which is called magnetic resonance spectroscopy and detecting the level of FU that is going on. And then you probably inject

some intervention like interferon alpha and then also you looked at how it is modulating the FU activity.

So in one case you are just putting FU looking at the signal that is coming from the patient then subsequently you are dosing with interferon alpha and looking at the activity of FU, how it is changing. So these experiments were done. So for a patient treated with 5-FU, F19 spectrum of the liver metastasis from colorectal cancer were collected. You can see the signal that is coming 5FU and some catabolites that is coming and after treatment the catabolites that came out was α-fluoro-β-alanine. So you can see here is the catabolites after some days, actually the catabolites that coming out prominently.

Now what next step was done? Interferon alpha was given to that patient, and looked at what kind of response was seen. So 5-fluorouracil plus interferon alpha was given and you can see here was the anabolites that started and you are getting the 5-fluorouracil plus catabolites. So you are essentially saying that how the intervention changes the metabolism in a quantitative manner if you are detecting these signals coming from the patients. So this is the good part of doing the drug metabolism in vivo. So one can detect what signals comes out and what is the pathophysiology or all those will be understood by doctor. So it helps actually medical practitioner to understand the metabolism that goes on in a patient and they can tune their drug dosage or drug regime for a patient.

So that is what it helps actually understanding the path that drug will take, how metabolism is getting affected in a non-invasive manner. So essentially patient is going in this machine, underdoing the MRS and giving you the response from the localized place of liver where metabolism happens and then doctors can decide what needs to be done in case of the altered metabolism. So that is a good part. Now this in vivo detection is called MRS. So you can put the patient in MRI scanner and then do the MRS magnetic resonance spectroscopy you can detect various metabolites that are coming.

So here I am showing you from brain various metabolites like a choline, keratinine, GABA, glutamate all these signals can be detected. So this gives us confidence that anything that changes in brain, we can do localized spectroscopy called MRS and detect what kind of altered metabolism we have. So I will give you some example of altered metabolism, which can be detected using MRS. So what essentially it is done? One has to put the whole organism or whole animal in the magnet, and you apply some magnetic field – NMR radio

frequency on this say rat. In the magnet, without any magnetic field all spins are randomly oriented, and with the magnet, spins are now aligned and that gives you the signal. That is what we know from this course, that whatever being observed in the magnet and that gives us signal. So if you are putting whole rat and taking the slice of his brain, like a slice means like a voxel or something like that. In MRI scanner getting the signal exclusively coming from there, one can know what is going on the rat brain or even for any organism.

So, one can combine this MRI with MRS, MRI gives you image, this is not a course for MRI, but just give you a very brief idea, you can get the magnetic mapping of the brain using MRI magnetic resonance imaging. So you can get the image and from the section of that image, if you do the MRS magnetic resonance spectroscopy, similar experiment that we did that we had discussed in this course. Take the signal, do the Fourier transform, you get the MRS spectrum. Suppose, from this section of the brain, you can get all the metabolites that are coming like choline, glutamate and all those. And now, this actually is essentially used in the drug metabolism. So if this is a normal patient, this is the response.

If some drug has reached here, the response will change and that is what we will be detecting. So MRI and MRS combined gives the whole magnetic mapping of the body and what kind of metabolism happens. Similar thing can be done say P31 MRS of the human liver, if you take the human liver, take a phantom and transverse MRI around the liver you can get various phosphorus containing compounds like beta ATP, alpha ATP, gamma ATP, NADPH, all these signals will be there, phosphocholine, phosphoester, PI, many of these can be detected. Now suppose I am giving an example, so suppose, one person is exercising very vigorously and you take the MRS of that person. So some of these energy currency will be metabolized.

So you will see the response or the ATP concentration will be going down. Suppose you want to give a drug and look at the metabolism of that, what will happen? So, suppose this drug contains phosphorous or it enhances the energy metabolism. You will see that the ATP currency, the energy currency like ATP, ADP, their concentration will be going down. So that means if you combine this in vivo detection, one can find it out how the concentration of each of these metabolites are changing in case of exercise, in case of drug administration and all those. So combining the in vivo detection of these signals helps us in understanding the metabolism as well.

Another example I want to show you what happens in rat brain. So you can see the rat brain which has a seizures, you can see lots of this energy currency showing very high signal compared to the baseline. One can have that these concentration or these signals looks slightly higher in case of the seizures brain. So, that means brain becomes hyperactive and this I have taken from Patel et al., Journal of Neuroscience. So you can see lots of signals seems to be hyperactive, some of those are however lower but many of these seems to be altered.

So you can integrate these peaks, find it out which of the phosphorus containing energy currency becomes hypermetabolites in case of seizure brain, and this was done using MRS. So, if you administer a drug, and to look at how the drug impact on the activity of the brain, you again record a spectrum, and look at the energy currency and the signals coming from the spectrum, that helps us to understand what drug did to the brain. Although you are not detecting directly the drug metabolites, but we are looking at the response of that drug on the metabolism of this energy currency. So that is how you can use the in vivo detection of the metabolites to understand the drug metabolism. Now another example I am showing you for 1H MR spectroscopy for an A-beta containing mice.

A-beta is a protein that aggregates in the Alzheimer's brain. So, the mice was basically induced with Alzheimer's and to look at some of the signal that came with a wild type and the Alzheimer's type of brain, taken a section, and did the MRS, and if you look at some of the signals that were different in the two spectra like a glutamate was same, NAA was same, but look at some of these seems to be different. So, what it shows is that in case of Alzheimer's, the metabolites that are present in the brain are different. This gives a very useful insight how actually disease changes the metabolic state of the brain, and that essentially are used in understanding the altered metabolism in case of disease.

So here one can see it in vivo NMR spectrum of a cerebral cortex in the mice that are affected by the Alzheimer's. One can see the concentration of these seems to be lower. Here again it seems to be lower. So differential concentration of metabolites gives us an important insight of altered metabolism in the rat brain. Now rat was smaller so you can put directly in the magnet.

For human it is little bit difficult, but that gives an idea how your drug regime or drug dosing should change. So this study on animals will be very crucial in understanding how

we can translate this idea into the human. So, at the end I would just want to conclude in this week we started from understanding the drug design and then we went ahead and looked at how the NMR can guide us in having a better fragment based drug design, pharmacophore based drug design, various drug design. Once the drug we have designed just we wanted to explore whether the drug is effective, how metabolism is impacted and how NMR can help in understanding the drug metabolism. So slowly we went into the drug metabolism study where we looked at the in vitro part how we can use the body fluids to quantify the drug metabolism. And today I just briefly touched upon how we can combine the in vivo approach for understanding the drug metabolism.

This part of the NMR is called MRS magnetic resonance spectroscopy, which helps in understanding the drug metabolism in vivo. This is a field in itself, if you are interested, go and explore this. This is a beautiful field to understand the drug metabolism or metabolism in general without doing any surgery. So for in vivo studies, in non-invasive manner this beautiful concept of MRS can be used for understanding what is happening in body. So the next week we are transitioning into the another aspects of a structural biology in NMR called solid state NMR and that is essentially whatever protein cannot be solubilized, neither crystallized, that will be looked in using solid state NMR.

So transitioning from the liquid state to solid state will be done next week and that is going to be last week for this course. So looking forward to have you in the next week course, which is solid state NMR for biological molecules. So thank you very much and looking forward to have some exciting questions from you and a vibrant engagement with you during the live session. Thank you very much.