NMR spectroscopy for Structural Biology
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Lecture: 48
NMR in Drug metabolism I

Students, good morning. So this week we are discussing NMR in drug discovery. In the last lecture, essentially I discussed how we can use NMR to develop a drug, starting from how we can use the NMR in SAR structure activity relationship. Then looking at the binding site on the protein we can start developing the drug. So we can find another binding site if possible. Then attach these two ligands to have a better binder. I gave you few of the example how we can start from say millimolar binding strength, *K*d and all the way by joining this fragment we can go up to nanomolar.

So this is called fragment based drug design. Then we also looked at how we can look at the ligand side, looking at the interligand NOE and then again, we can join these ligands to make a better binder. So this is kind of a development. First is like we have identified a binder by either detecting on a ligand or detecting on a protein.

So once we identify the binder we want to make it better binder combining the medicinal chemistry approach and the structural information that we are getting from NMR spectroscopy. So can we combine these two to develop a better drug, a stronger binder, a more potent drug and that is what we were discussing, how NMR is helping us in doing this. So actually, you can measure the NOE between two ligands that are binding at two different sites, and probably looking at what are the proximal atoms that are interacting through this NOE, and then one can join them to have a better binder. So that is what we looked at and then I gave you few of the example how we can use these concepts of SAR and then ligand-based, fragment-based drug design to find a better binder.

So we ended it there, now we will move ahead and we will say that once we design the drug and discover the drug, we have to look at what this drug is doing. Where it is going in the body? Can we detect whether it going to the right site? How NMR can play a role? We are going to now slowly delve into these concepts of drug metabolism and detecting the drug and its metabolites in the body using either in vitro approach or in vivo approach and let us see can we detect it and then this will help us to knowing the efficacy, potency

of a drug, how drug travels through the body. So many other aspects can be explored if we combine the concept of drug metabolism and detecting by NMR in per se. So, let us move ahead with the concepts.

So, you know from a basic course in the drug discovery and design that the basic property of drug should be something called ADME, drug should be absorbed in the body. Any drug that you are taking, whether you are taking an oral drug or injectable drug or subcutaneous drug or intravenous drug, any of these drugs whatever the mode of injection it is, it has to be absorbed in the body. The first and most important thing is absorption. Once it is absorbed, it has to distribute throughout the body. So distribution should happen as systemic distribution.

It should reach to the location where it has to act. As many of the drug we take through oral route. So when we take a tablet or capsule, it has to get absorbed in our system. Then it should be distributed and it should reach to the point of action.

So that is a distribution. After that, once the action is done, this drug is not from our body, it is a foreign particle, xenobiotic. So it has to be metabolized, it should be degraded into smaller pieces and then finally it should be removed from the body. This is called ADME. We are actually required to understand the toxicology and this field is called toxicology that is studied in human or animals.

So it should not produce any toxic effect. That is the primary requirement. We are taking a medicine for being cured. It should not produce toxic things so you have to identify whatever metabolites are produced by the drug metabolism should not have any or minimal toxic effect. We cannot ensure for no toxic effect but should be very minimal toxic effect, otherwise drug has to be recalled and that drug cannot pass through.

So, these four points has to be very well taken care, it has to be absorbed, distributed, metabolized and excreted that is called ADME. So, here in the cartoon representation, so first thing is like you can even take it liberation, the active pharmaceutical ingredient should be liberated from the formulated capsule or a tablet or wherever it is. And then it comes to our body by absorption, it gets absorbed, it distributes through our circulation system and that is a distribution, it reaches to brain, reaches to head, reaches to hand, all those. And

then metabolism, so it should be metabolized and finally it should be excreted through urine or through feces or somewhere or sweat, but it has to go out of the body. That is the typical property of any drug.

So this whole concept is called pharmacokinetics. The kinetics of a pharmaceutical molecule, pharmacokinetics that is what this study is. If you design a drug, you are administering drug, how it is going through our body. So you are taking a medicine suppose through oral route, it should go to your liver, to intestine and all those and then it should be absorbed. So it should be absorbed through your tissue and cells, how it will get absorbed then how it is broken down.

So most of the breaking down happens in liver. Let us look at it in little more detail how it happens and finally how it is distributed, where it goes, what are the transporters for these drug molecules. So here you can see there are various transporter and one of the obvious transporter is a blood. So blood goes everywhere. So blood can take drugs throughout the body and finally after metabolism it should get excreted. So that is total concept of these drugs, journey from the administration to the excretion is called pharmacokinetic.

So drug metabolism and how it can vary among the patients in a drug response that determines lots of things. So when you go to a doctor, a physician, he prescribe drugs based on the characteristic of a medicine. And the probability that has been obtained from reliable and reproducible clinical effect. What result it will produce, that is the basic concept that physician has.

There are some already reliable and reproducible results obtained in a clinical trial that this drug should act in this particular manner with some variation and that knowledge doctor has, physician has. When one new person goes to physician. He understand the characteristic of a drug and the probability that has obtained from clinical trial, so he prescribe a drug, but as you know all of us are not similar. Our system response is different, therefore it is expected that there will be difference in the drug response among the patients that goes to a physician and that is obvious, that is common, because we are different. Genetically we may be same but our protein expression level, transcriptomics, like transcript level we are different or protein may be same but their action like how fast or how slow or how pronounced they work are different.

The drug response in different patient can be different and that often leads to challenge in optimizing a dose, how much dose should be given to particular individual. Depending upon the severity of the disease, one dose will suit to a person, another dose may suit to another person. Nowadays, what is happening that generally doctors prescribe one dose for adults, one dose for child. If you know from your common sense, child has a lower dose, adults have one dose. But let us talk even adults, adults like all adults are not same, so it is intuitive that one has to prescribe the medicine or a dose should be person specific rather than a like a blanket prescription.

Now how to get that person specific response that is called the field called personalized medicine. So every person should be given in a particular dose form and that can come only if we have a detailed study how a person is behaving for a particular kind of metabolism, drug metabolism. So that will dictate about the dosage, the particular kind of medications should be given to a person. Now for doing that actually lot more studies required a larger sample size has to be taken, person specific dose should be determined looking at the person metabolism.

And therefore drug metabolism becomes of paramount importance for designing and optimizing the drug response in a particular person. Then another major hurdle that major drugs are effective in only say 25 to 60 percent of the patients. It is not effective in some patients. Now this is the question one can ask, why? Drugs should, like all of us are human, so should be effective on all of us.

But that does not happen. One drug works for a person fantastically. It does not work for another person. Now, that is the paradox still exists and therefore it has to be studied in detail and here comes the NMR, which can study person specific the drug metabolism by taking some of the body fluid. in vitro we can use the body fluid like a tissue extract or a urine or a blood serum or plasma or one can even take the intact cells or biopsy or perfused organ. NMR can even offer to study the metabolism in whole animal model or human subjects.

Combining probably MRS and MRI, MRS is magnetic resonance spectroscopy and MRI is magnetic resonance imaging, you can even look at the fate of a drug inside the body. So this is the developing field. But in vitro can be done quite easily taking urine, taking serum, plasma or tissue extract, biopsy sample and all those and it offers the unique probability.

NMR offers the unique ability to do these kind of study in vitro and in vivo simultaneously. So without perturbation or taking just body fluid, without changing much in the body fluid, you can detect what is the fate of a drug that is administered.

And that will create a wealth of information that leads to the personalized medicine that is the future subject in the medicine field. How you can tune particular drug or a dose for a particular patient? That is going to come in future. So what happens to the drug? So when drug enters to the system? One thing I said that its availability is very low. So why it is low? What happens? When it go to like liver, the proteins are there, which starts cleaving, and there is a heme protein called cytochrome P450 that plays a key role in the metabolism of drug or any xenobiotic.

This is a heme protein. This is the structure of a protein. There is a channel here, here is the N-terminus, the prosthetic group where actually heme binds and here is the C-terminus. So this is the protein cytochrome P450. It is an important protein, it also involved in biosynthesis and degradation of endogenous component such as steroid, lipids or vitamins. It is also responsible for metabolizing any xenobiotic, drug that we are taking.

What can happen? The P450, cytochrome P450 action can be differential in a different person. Therefore, we ask the question why one drug is effective in one person, it is not effective in another person, can be understood as the protein expression can be different in different person. Therefore, the available drug that is there can be different because of action of this protein called cytochrome P450. So cytochrome P450 plays an important role in chemical toxicology. So this is the structure, it is a heme binding protein, you can see the profile, the heme binding site and in an oxidation reduction reactions coupled with NADPH cytochrome P450 actually does couple of oxidation reduction and that helps in metabolizing any xenobiotic.

Interestingly in humans, there are 54 cytochrome P450 genes and they are responsible for different kind of metabolism. Today we will be highlighting some of those. So in liver which is a major site of the metabolism, this cytochrome P450 exist. So this protein cytochrome P450 actually start metabolizing drug and as we know that once you take this drug it goes and goes through liver. Here is our metabolism happening and then it goes for absorption also and at this place our cytochrome P450 is sitting.

So cytochrome P450 mediated metabolism happens, but in erythrocyte, RBC or even in the epithelial cell of small intestine. Metabolism can happen in our liver, it can happen in intestine also, these are the important site for drug metabolism. Like one of the cytochrome P450 is CYP3A which is found in erythrocyte. RBC structure is here. This also can do metabolism. So you can see first is liver, it can be in RBC, it can be in intestine, epithelial cells of intestine, these are the potential site where drug can be metabolized.

So about 40 percent of commonly used drug is only available because that gets metabolized by limited absorption or first pass metabolism. First of all metabolism means it has to pass through metabolism and then it can be absorbed. So now the drug whatever we are taking is not 100% available. It gets metabolized.

How much? Depends upon a person. Where it gets metabolized? In liver, in RBC, in small intestine. So here is the first metabolism that happens after oral administration of a drug. So for this I have taken from New England Journal of Medicine, you can see if you are taking the medicine through oral administration, it is entering to the liver, so this drug is called Felodipine which is actually used for treating the high blood pressure. So essentially this drug is a calcium channel blocker and what it does? It works by relaxing the blood vessel, so heart does not have to pump very hard and that is how it ease out the blood pressure. So this drug is taken, suppose this drug we are taking 100 percent Felodipine, this is going through the liver.

Now here is liver then goes to a small intestine and all those and it gets metabolized. So, here, this drug is going 100 %. After metabolism here in RBC, we have this protein called CY34a, it starts getting metabolized and then if you look at the drug percentage that starts getting reduced from 100 %, here is a 45 %, here 15 %, 13 % or so. Somewhere it is 19 % so in gut lumen, in erythrocyte this is 90 % or so. Finally, when it comes out and goes for a circulation it is 15 % or 45 %. So, rest about 55 % is lost in the metabolism

in liver, small intestine and RBC. Now that is called bioavailability, how much drug is available for circulation and that has to reach to the point of action. So 100% it starts and you can see that about 55% to 85% it gets lost, it gets metabolized. So if you take it, you are taking some 100 %, but it is available only 15 to 20 %. Now that requires investigation in a person specific manner. So the action of these proteins can be differential in different person.

So you cannot recommend or prescribe one dose for all, it will not fit. So for one person it will be very effective, for another person it will not be so effective. So probably you need to readjust the dose so that that person also gets the significant amount of bioavailable drug to reduce the blood pressure and therefore you see the doctor keep changing the dose. So looking at the response from you, they keep changing the dose. It can start from lower dose, then depending upon how you behave, they can increase or decrease the dose. If required they increase, if required they can decrease the dose and that is the iterative process goes on and that is determined by the bioavailability of a drug.

So here is the detailed description of the same. We are starting from 100 % and then in some of the tissue, erythrocyte and the intestinal tissue, it gets metabolized and finally we have only 45 %. So some of the commonly used drugs, which has a low bioavailablity. You know many of these drugs and what are the metabolizing enzymes, and what is the percentage bioavailable, I am showing you. So one of the drugs say let us say Aspirin, many of us take for headache. Aspirin, enzyme is called esterase and you know it is a easily soluble drug.

So if you put it in the water, it is easily soluble, you take it through oral or you dissolve in a water, just drink the dissolved water, it is 68 percent, one of the higher one. Now another one you can choose any of these drugs Felodopine, that we just now we looked at so only 15 %, it is a heart disease drug. There are many other drugs diclofenac, these enzymes that are involved and 54 percent. So if you look at many of these drugs, the bioavailable active pharmaceutical ingredient is about 50 % or less than that. So one important area of research that is coming up how you can increase the bioavailability of a drug.

For that also one has to do a systematic investigation looking at what is the fate of a drug, what is the fate of metabolism that happens and how much drug is available for action. So the active area of research to increase the bioavailability, how we can increase the bioavailability. Now these guys, the paper that I am citing it, actually they come up with an interesting thing. So like a consequence of inhibition of first pass metabolism, if you do inhibit the first pass metabolism, where our cytochrome P450 is sitting, if you inhibit that, you can increase the bioavailability.

So for the same drug that we were discussing, the Felodipine, If it is taken just in water, you look at the bioavailable, this is the time here in hours and this is the percentage or the plasma concentration that is reaching to the blood, plasma concentration in nanomole per liter. So if you look at it increases 0 hour, when you take the drug, with time it increases goes to about 15 percent, and then slowly slowly decreases in 8 hours, it becomes about 1 percent or even less than that. But if you take this drug with a grapefruit juice that inhibits the first pass metabolism and you can increase the availability of this drug up to like about 30 percent and even after 8 hours this remains about whatever we had earlier 8 to 10 percent. Now just by taking this drug with a grapefruit juice, there can be another substitute but this paper describes about grapefruit juice. Now how that response happens because finally you are giving and increasing the bioavailability, does it changes the heart rate? So if you look at here heart rate plotted bit per minute.

So how hard you are pumping? Because there was a pressure on the heart that is why you are taking the blood pressure drug. So if you look at the grapefruit juice and water in 8 hours the grapefruit juice is slightly behaving better than the water and even after 8 hours both are doing fine. So I think you have improved bioavailability and that is how heart rate is slightly performing better when you are taking with a grapefruit juice. Another parameter is blood pressure, does it maintain the blood pressure? So actually if you look at the grapefruit juice actually it reduces blood pressure, it brings in the range of 115 rather than going to more than 120. So grapefruit juice not only increases the bioavailability but also it decreases the heart rate

and blood pressure in mm. So, both the blood pressure, basically systolic and diastolic actually improves, when it is taken with grapefruit juice. So blood pressure and heart rate were measured while patient is standing, and not sleeping. So in an active condition measured and what they found that when this drug is taken with a grapefruit juice, essentially it increased the bioavailability of a drug, because it inhibited the first pass metabolism and that is how the effective concentration is there. Now the question is how this was measured? So one of the analytical techniques could be used for measuring it and that is what we are going to discuss how NMR can play a role in measuring the effective concentration of a drug.

Now so what actually cytochrome 3A4 mediated metabolism did to drug substrate? So when you take any of this drug, it goes and binds to some of the protein complexes, co-activator comes and there is a co-repressor, this is our CY34A and that basically plays and role in the metabolism. Now once you take a kind of an inhibitor that probably inhibits, so

that can decrease the activity of one of these drugs and essentially, if you inhibit or reduces the activity of this protein, then the bioavailability can be increased. So many of such things can be tuned for increasing the bioavailability or you can formulate your drug with something else. Now active research is going on how to co-formulate your drug with something else which increases the bioavailability.

So I will stop it here. So on the metabolism part, if you look at the plasma Felodipine concentration in nanomoles per liter, you can see the patient, in a healthy subject, you can see the concentration of plasma Felodipine was quite higher. The patient who has increased activity of this particular enzyme, cytochrome P3A4, the concentration of this drug was very less. It is one nanomole and here we have 6. So that means the person who has a more active protein of CYP3A4, actually the drug is not that effective and in healthy subject it is quite a bit. So what we need to do now, if you want to really treat the heart disease, the blood pressure or something, you have to temper with this enzyme activity. So that whatever drug is given, it is not metabolized and the effective concentration should reach to the point of action so that heart does not have to overact. So, that is what it is done by grapefruit juice and these all information I had taken from this prestigious Journal, New England Journal of Medicine, you can refer to this Journal.

So, this is called co-formulation and increasing the bioavailability. In the next class what I am going to discuss with you, how NMR can play a crucial role in understanding the drug metabolism at various stage using in vitro and in vivo approach. So let us first start with in vitro approach and then we can go to in vivo approach. I will stop it here and looking forward to have your questions in the next class. Thank you very much.