**Organic Chemistry in Biology and Drug Development**

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**Lecture - 62**
**Pharmacokinetics and Pharmacodynamics**

Welcome back to this course on Organic Chemistry in Biology and Drug Development. In the last 21 lectures, we were discussing the drug development processes. At first, we discussed about the modern methodology to design a drug rationally and then develop it for the market.

Then we went for rapid screening of library of compounds. Then we went for different case studies of various diseases and what is the reason for the onset of the disease. Once the target is known you can find molecules which are acting as drugs against those targets. We have taken a various types of diseases like the problems associated with the neurotransmission, then the anti-cancer, anti-biotics, anti-ulcer, anti-viral.

During this journey, we have briefly described one important aspect of drug discovery program. Once the target is identified you do some in silico screening of those molecules. Before going to the human trial *i.e.* clinical trial, there is something called preclinical trial. In preclinical trial phase, the drug which is has the potential you have to check its pharmacokinetics and pharmacodynamics property which are abbreviated as PK PD studies.

We are going to discuss what are this PK PD and how they can be determined. Once the drug crosses through this PK PD barrier then only it can go to the next level that is the clinical trial. So, this is basically preclinical studies and PK PD is a very important aspect. Now, we have two terms- one is the pharmacokinetics and the other term is pharmacodynamics.

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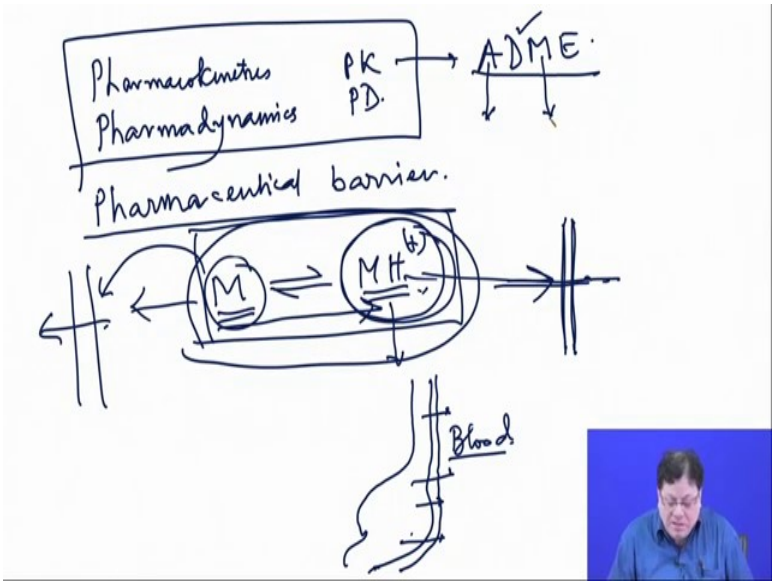


Now, these are abbreviated as PK PD studies. If you remember these two terms PK is basically the pharmacokinetics. Pharmacokinetics is what the body does to the drug and pharmacodynamics is what the drug causes to the body. It is basically mutually exclusive.

Usually for taking drug, the oral route is preferred than the other intravenous IV route simply because the oral route is simpler no pain nothing. If a drug is taken by the oral route it has to survive in presence of the enzymes that are present on its passage through the gastrointestinal tract.

We know that stomach is highly acidic. So, there are many barriers that the drug has to pass initially. The drug is either encapsulated in a pill or in a capsule kind of thing or it could be used as a tablet where various binding agents are given to the drug.

So, the first test for a drug is that when it goes into the GI tract. The drug has to be released. It has to be stable to pH. It has to be stable to whatever enzymes are there and then it should be absorbed from the GI tract and goes into the blood stream. Now, pharmacokinetics basically starts from the absorption stage. The next steps are- distribution, metabolism and excretion.

These are the 4 things which fall under pharmacokinetics. Before absorption, it has to withstand the acidity of the stomach and many of the enzymes present in the GI tract. So,

that is called the pharmaceutical barrier. So, initial barrier is not PK PD, pharmaceutical barrier.

I give you one example like when penicillin. The first penicillin that was made and was penicillin G *i.e.* the benzylpenicillin and penicillin is extremely unstable to the acidic pH of the stomach. So, it could not be given through the oral route. There are many drugs which ca not given orally because they are not stable under those conditions and the conditions are prevailing in the GI tract.

In those days, penicillin was given intravenously but later on lot of other penicillins have been made which are stable to those conditions. The pH of the body varies from different parts but mainly it is maintained between 6 to 8. Like in the blood it is about 7.4, something like the slightly alkaline.

There are other areas where it could be mildly acidic. A drug should have a $pk_a$ between 6 to 8. Once the drug has passed the pharmaceutical barrier *i.e.* the pharmaceutical screening test it is stable under the condition in the GI tract.

Then we have to think about the the absorption of drug. If it is too polar it will be more soluble in water. It will have a difficulty to cross the cell membrane which is hydrophobic in nature. On the other hand, if it is too non-polar *i.e.* hydrophobic then it will be dissolved as droplets in fats. If fat has the ability to dissolve forming the kind of a micelle the fatty compound is the hydrophobic drug inside.

There will be no question of distribution absorption through the membrane. It should not be very lipophilic. On the other hand, it should not be highly polar. If you have a molecule M it will be convenient to exist in equilibrium between a polar form and a non-polar form.

When it is required the drug has to have some polar character to cross membrane. It will be $MH^+$ which will cross that barrier. It could be some organ barrier like GI tract barrier because it has to be absorbed there. If it is required that ionic species it will cross through the barrier and the whole equilibrium will be shifted to this direction.

On the other hand, If the barrier that is present to this system to is hydrophobic the non-polar molecule will pass through that non-polar membrane. And then what will happen?

That $MH^+$ plus will move on the left side. So, basically this is a good option that you should have a molecule which has got some ionic character.

Usually the nitrogen containing molecules exists because the nitrogen containing molecules have a $pk_a$ between 6 to 8.

Both the species will be present at pH 6 to 8 in the different locations of the human body. If we now look at the number of drugs and then screen them structurally you will see that most of the drugs contain nitrogen. The main reason is that it can actually distribute itself into an ionic and the non-polar form.

This is your GI tract, this is the stomach and then you have the large intestine, the small intestine and the drug has to be absorbed. It could be absorbed from esophagus, mouth. Many drugs are actually absorbed through the mouth that is the saliva. The whole drug absorption process is basically the entire GI tract. It starts from mouth and then ends into the small intestine.

Once it is absorbed it goes into the bloodstream and then it goes to the bloodstream. There is a checking point because anything which is foreign to the body, the body wants to reject it. So, the body sees that some molecule has entered which is a foreign molecule.

The body wants to expel it from the body. So, it is called metabolism. If the drug is highly water soluble, highly polar the body does not have to do much because it is already soluble in water. It can be excreted through the kidney, very easily.

There has to be a balance between the polarity and the non-polarity. The drug has to be made water soluble before the body can excrete it and it is called metabolic. So, one is absorption i.e. the drug has to be absorbed in the GI tract and the drug has to have some balance between the hydrophobicity and the lipophilicity. Then there is distribution.

Distribution is basically comes to the blood stream and wherever the blood is distributed the molecules go there. There are also some molecules which are present in the blood that may not allow the drug to reach the target because ultimate aim is to be absorbed and then to be distributed.

Before reaching the target there are some proteins which are called albumins. They are very non-specific. Many molecules can bind or provide surface where the drug molecules can bind. Human serum albumin sometimes absorbs the drug and then the drug cannot reach the target. So, distribution is also important whether it is really evenly distributed and then the drug has to reach the target.

If albumin is absorbing the drug you take another molecule which has got more affinity for the human serum albumin. If you take both the molecules together your drug will not be preferentially absorbed because the other molecule has more absorption potential.

This combination of drugs is used to stop the actual drug from binding to the serum albumin. So, that is absorbed distribution. Next the metabolism comes.

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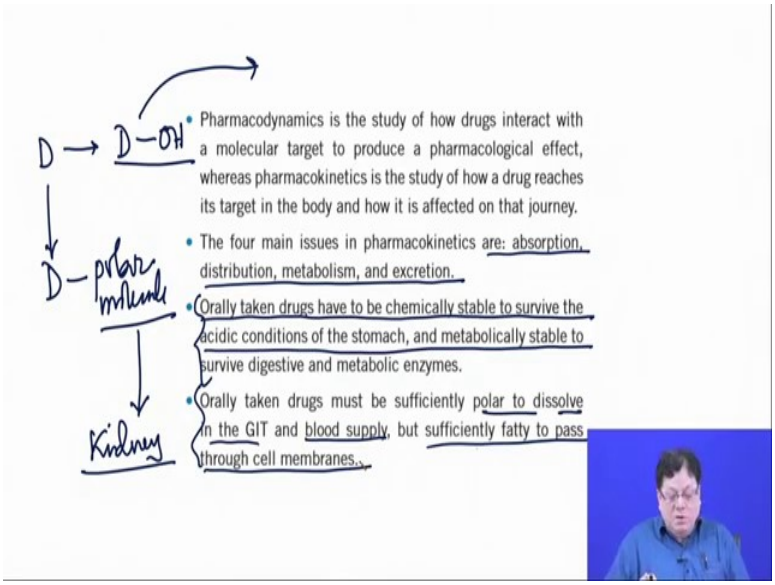


Drugs have to be metabolized because the body treats that as a foreign molecule as most of the drugs are not highly soluble in water. So, they have to be made soluble in water. There are two ways to make the drugs an water soluble fragment. One is putting hydroxyl groups in the molecule because you know hydroxyl groups enhance the polarity.

But the question is why hydroxyl, why not amine? Amines are also water soluble but we have the oxygen source and we have the enzyme system to oxidize different molecules utilizing the oxygen.

The other way that is called phase I metabolism and another phase of metabolism is there where the drug is conjugated to a very polar molecule. So, there are two ways-one is the drug can be converted into OH or the drug can be conjugated to a polar molecule and once this is done then they can be excreted mainly through kidney because now they are water soluble unless the molecular weight is very high. Then there is a problem that kidney cannot filter that.

Pharmacokinetics has 4 steps absorption, distribution, metabolism, excretion. When we talk about this pharmaceutical test that where it survives the acidic conditions of the stomach that is only for orally act active drugs, not for the drugs which are taken intravenously.

There is a way to bypass that pharmaceutical problem because you can directly inject to the blood. Orally taken drugs must be sufficiently polar to dissolve in the GI tract and into the blood supply. It should be sufficiently lipophilic to pass through the cell membranes.

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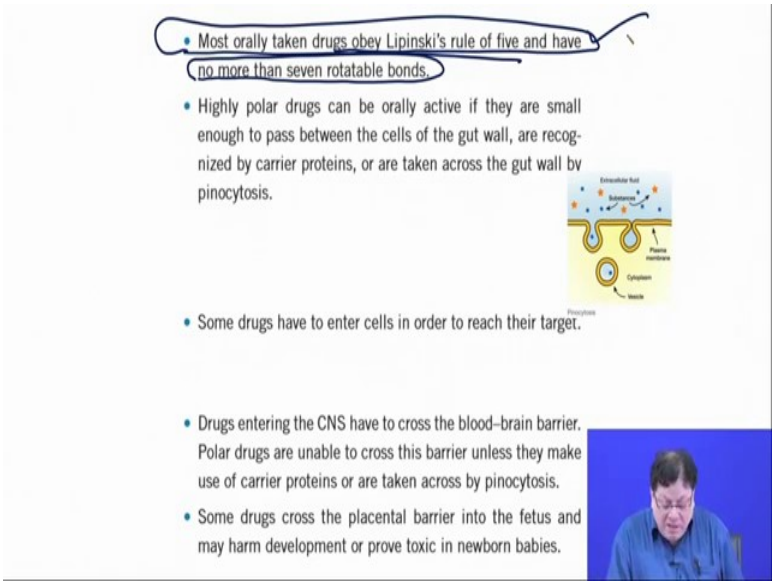


Now, I said that there are two ways of doing the metabolic changes in the drug. That is actually basically absorption- how the drugs are absorbed and then distributed. You must follow Lipinski's rule of 5. Molecular weight of drugs are less than 500, there is 5 hydrogen bond donors, 10 hydrogen bond acceptors and Log p value less than 5, not more than 7 rotatable bonds.

It has been found that they have a very good absorption as well as solubility in the blood. Now, these are not sacrosanct. These are not written in bible. This is basically a first preliminary screening test. The pharmaceutical companies do not have to screen out lot of molecules to synthesize.

So, the industries follow the Lipinski's rule. We are going to discuss the metabolism because that is where some organic chemistry is involved.

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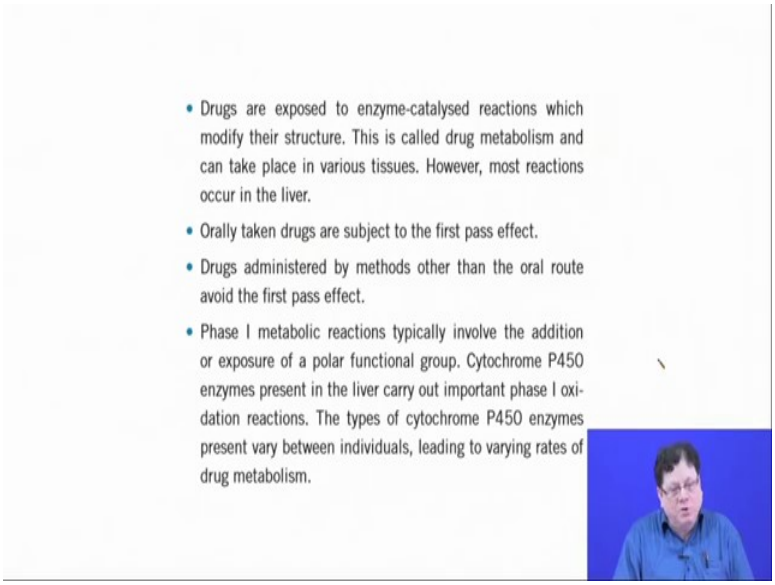


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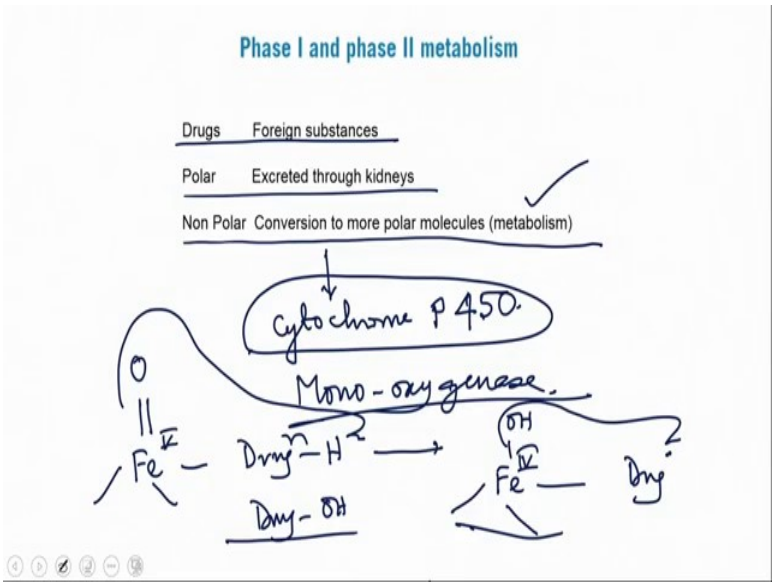

Foreign substances excreted through kidneys and it is called metabolism. But remember one thing the drug is given for the purpose of treatment of a disease. If it is metabolized very rapidly your drug is going out from the system very quickly. If metabolism is quicker than reaching the target then the drug will be useless.

You have to increase the dose but then you have to again be careful that whether you are crossing the therapeutic index and all other issues. There is another big topic which is called bioavailability that means how long the drug exists in the bloodstream. If you are prescribed penicillin molecule you take one in the morning and take another after a gap of about 12 hours. It has been found that the drug is sufficiently made available to the target for about 12 hours.

You take the next dose again. Some drugs have very good bioavailability. You take the pill only once like the hypertensive, antihypertensive drugs, diabetes drugs because they are more stable metabolically. So, it has to have good metabolic stability.

However, it should also flush out because many of these drugs are not very healthy for the kidney. They should be flushed out but they should not remain in the system after the disease is gone.

Once the drug enters into the bloodstream it crosses the first organ where a lot of this metabolic activities take place. Liver is our storehouse of many metabolic enzymes and one of the enzyme is called cytochrome P450. There is a wide range of the cytochrome P450 enzymes. They accept many substrates and they can hydroxylate very unreactive CH bonds. So, these are the classic examples of CH activation by nature.

In organic chemistry, these days the lots of CH activation chemistry are coming up and usually they use transition metals. This idea has evolved from this cytochrome P450 chemistry. Here they use the iron. How these iron ferryl oxo chemistry is utilized.

One is that oxidize the substrate and the oxygen comes out as water. In some cases, one oxygen atom is incorporated in the molecule and other oxygen atom goes as water. That is called mono monooxygenase and then dioxygenase means both the oxygen atoms are incorporated into the molecule.

So, cytochrome P450 has different types of activities- one type is monooxygenase because we need the hydroxylation. So, monooxygenase is basically an iron based enzyme. It is a ferryl oxo species like this.

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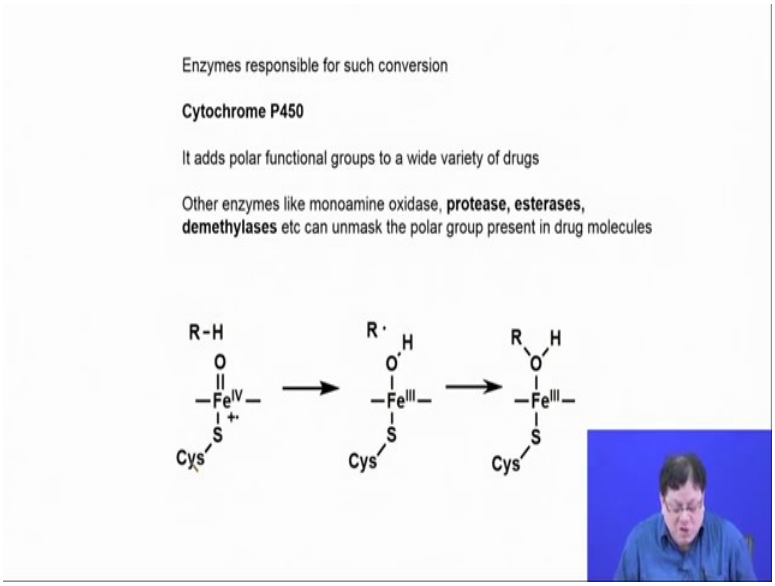


Different books have written the mechanism in different ways. Let me see whether I can simplify the whole thing. Once the ferryl oxo species is formed you have this drug which is attached to a H. The ferryl oxo will go to OH and this is one electron. So, it takes the hydrogen and the drug becomes the radical.

Whatever is the drug it has become a radical and OH. You have to be careful what the oxidation state of iron is. See one electron has gone here and iron gets back its electrons. Now the oxidation state is 4 and then this goes out and conjugates with the drug. The drug becomes hydroxylated and the iron now again goes back to 3.

So, that is the mechanism. We have seen this mechanism and we have seen that how this ferryl oxo species can carry out different types of reaction.

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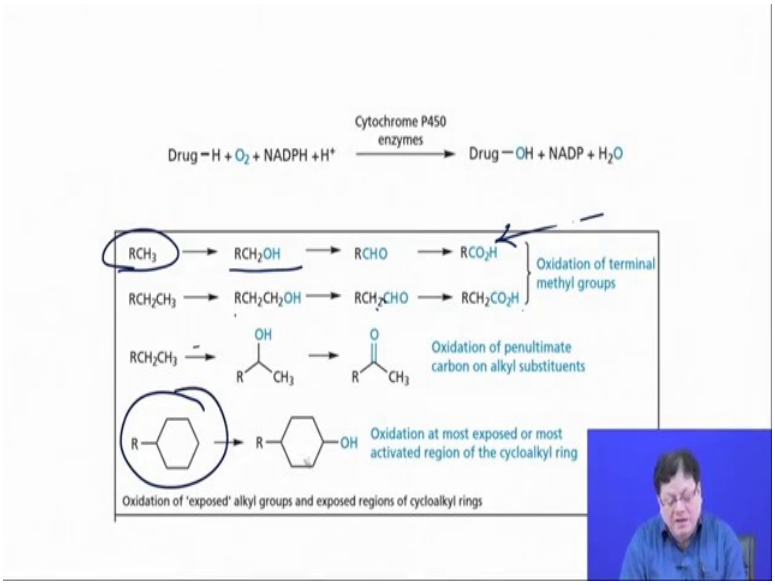


What types of functionalities are involved in this hydroxylation. $RCH_3$ can be converted to $RCH_2OH$. There are enzymes which can take it up to well up to the carboxylic acid because carboxylic acid is more polar or more soluble in water. Alcohol dehydrogenase converts it to aldehyde and then to the acid.

You can have cyclic systems like aromatic systems, benzylic which can be hydroxylated. Ketones can be hydroxylated *i.e.* alpha hydroxylation. Acetylenes will not be hydroxylated because propargylic hydrogen is the weakest bond.

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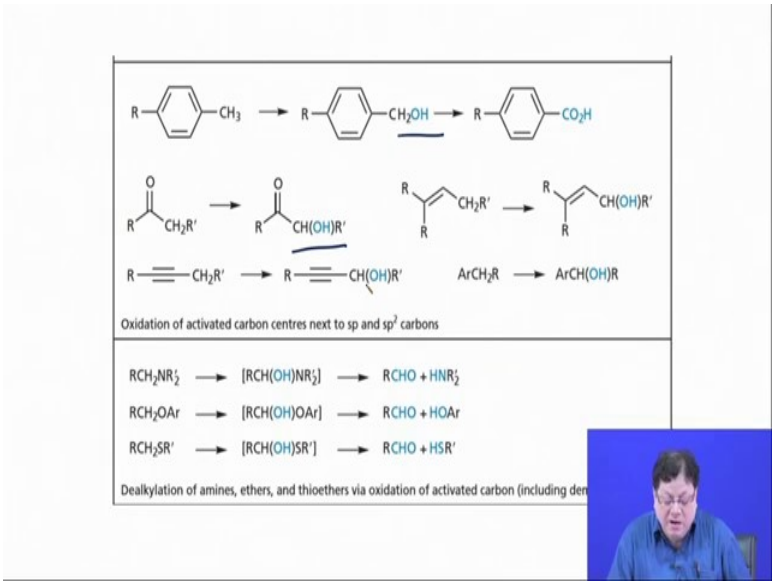

So, these are different types of hydroxylation reactions that are carried out in the liver by the cytochrome P450.

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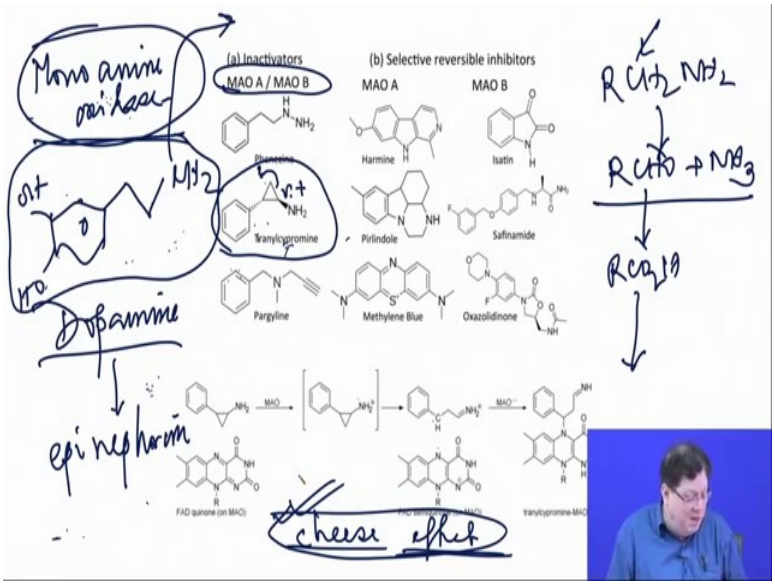


There is another enzyme which also converts or degrades the molecule into certain products which ultimately become water soluble. One is cytochrome P450. You have seen that in some cases after the hydroxylation they can be oxidized to the aldehyde followed by the acid by alcohol dehydrogenase enzymes and acids are soluble.

Another class of enzyme which are called monoamine oxidase. It takes monoamine means primary amine. It removes the nitrogen and forms the aldehyde.

There are many drugs which are based on dopamine. They have to be metabolized. Tyrosine goes to the DOPA and then DOPA goes to the dopamine by decarboxylation and that dopamine goes to norepinephrine and then finally, epinephrine.

So, dopamine concentration should be maintained at a particular level. Persons suffering from neuro diseases always check level of dopamine, level of serotonin, acetylcholine. Dopamine is forming from DOPA and usually it is converted into epinephrine. But there is an enzyme which is called monoamine oxidase.

It oxidizes any primary amine R $CH_2NH_2$ that goes to RCHO plus ammonia. Now, this is an oxidation reaction because this carbon was basically attached to one heteroatom. One

oxidation has taken place and then this can be converted into $RCO_2H$. If it is a drug $RCO_2H$ becomes water soluble.

However, this monoamine oxidase also becomes a target for developing drugs acting against neural disorders where the disorder is lack of the concentration or lowering of concentration of dopamine. The dopamine concentration can be lowered by converting to epinephrine. But epinephrine is another natural neurotransmitter. That is not a problem.

But when it is degraded by monoamine oxidase then dopamine is basically removed from the cycle of events (tyrosine goes to DOPA, DOPA goes to dopamine, then norepinephrine and then epinephrine). So, if dopamine is oxidized before it reaches this epinephrine or norepinephrine you have a lack of concentration of dopamine as well as norepinephrine and then epinephrine. So, that is a problem.

Monoamine oxidase takes out the dopamine from circulation and converts into the aldehyde. That is done by the monoamine oxidase. So, monoamine oxidase becomes a target for drug development also.

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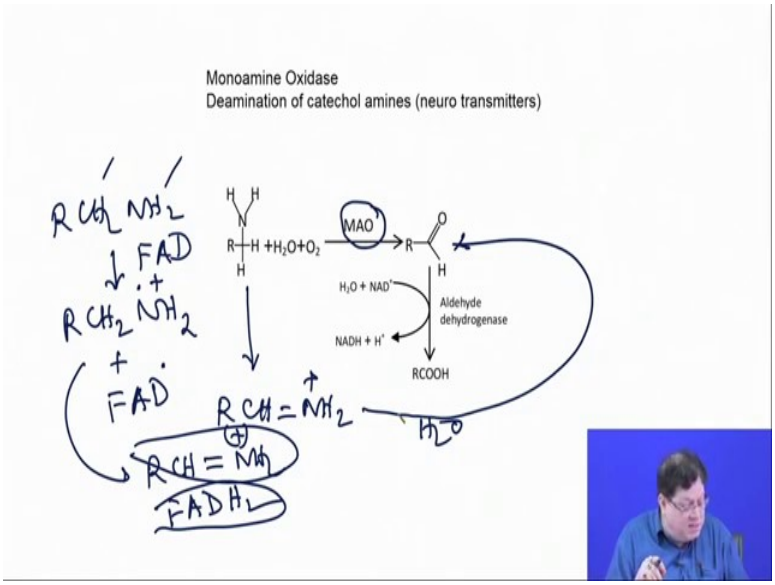


I will show the mechanism of monoamine oxidase. This is the monoamine $RCH_2NH_2$ and this is called MAO enzymes, monoamine oxidase. The amine goes to the ammonia and this is converted into aldehyde. This reaction requires oxygen and water. It first goes into an iminium ion and then water comes and hydrolyzes it to the aldehyde. It is one

electron type reaction. First, $RCH_2NH_2$ releases one electron. You have to oxidize it *i.e.* electrons have to now be released. Flavin Adenine Dinucleotide (FAD) takes up the electron and then it goes to R $CH_2NH_2$ dot plus. In the process, FAD goes to FAD dot. So one electron goes from the nitrogen because that most available. This FAD dot takes up the hydrogen from the alpha carbon and then that becomes a dot. These two dots combine and make this one the iminium salt.

Ultimately FAD becomes $FADH_2$. Two hydrogens have to be removed. That is the $FADH_2$ and this ultimately hydrolyzes into this.

So, monoamine oxidase is a very good target and some of the drugs have been shown here. One of the drug tranylcypromine is based on simple organic chemistry. It is an antidepressant drug. You can guess that once the nitrogen becomes positive radical that the radical can be established by putting a cyclopropane alpha to it.

It will open up like a radical clock. As it opens up the radical goes to some other place and that radical then attaches to the flavin at a different position. So, the whole co-factor is attached to the molecule. It is a kind of suicide inhibition. First electron is removed. But it is removed like an cyclopropyl methyl radical. Cyclopropyl amine radical also opens up immediately. So, there are many kinds of drugs.

There are two types of MAO inhibitors - MAO A, MAO B. If you want to increase the concentration of dopamine or even serotonin you have to use MAO inhibitors.

MAO inhibitors will basically inhibit the metabolism of any primary amine. Tyramine (decarboxylated product of tyrosine) is present in many food products and one of them is called cheese. Cheese has the highest level of tyramine. Many people suffering from neuro disorders are taking these MAO inhibitor. In foreign countries cheese is a very predominant item and then they were taking the cheese which contains lot of tyramine.

If tyramine level is increased in the blood that ultimately has a cascading effect leading to heart failure and this is called cheese effect. Cheese effect means a person who is taking antidepressant drugs based on MAO inhibitors. They should avoid cheese. However, medicinal chemistry has improved quite a lot. Now, we have selective inhibition of MAO A and MAO B.

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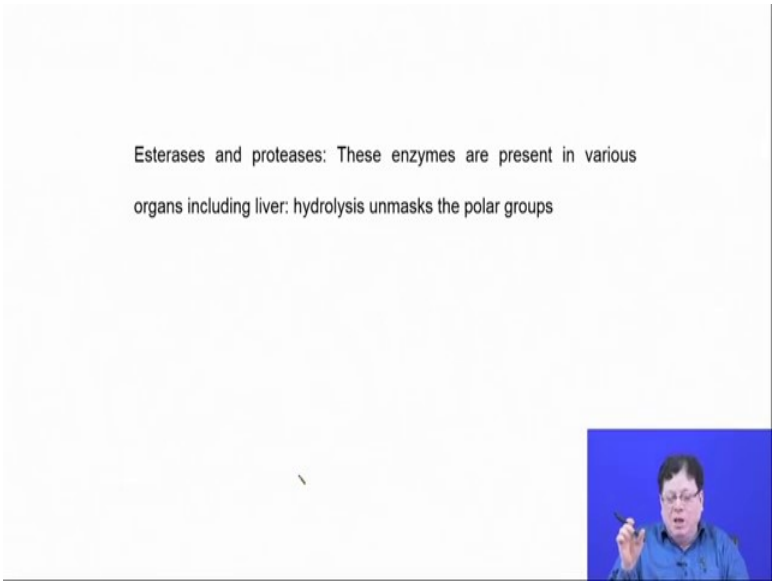


The second type is making the molecules more polar. In this case it will be bio-conjugation because you are basically attaching to a drug another biomolecule. What is that biomolecule? The biomolecule is nothing but a glucose derivative.

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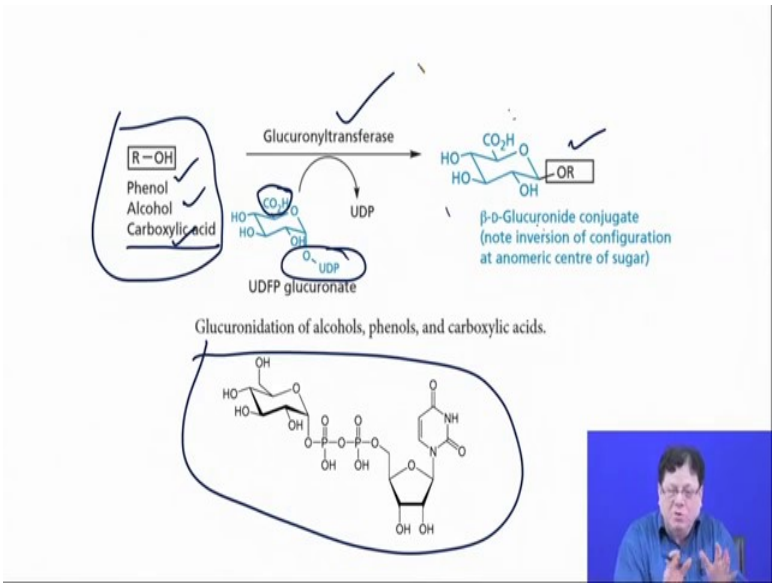


If you have an alcohol/carboxylic acid/phenol it undergoes glycosylation. Earlier it was the uridine diphosphate to make it a good leaving group. If you have ROH this forms the glycoside. If it is carboxylic acid it forms the O-glycosides. You can get N-glycosides,

C-glycosides, O-glycosides. The enzyme involved here is called a glucuronyl transferase because it is transferring that glucuronic group into the drug.

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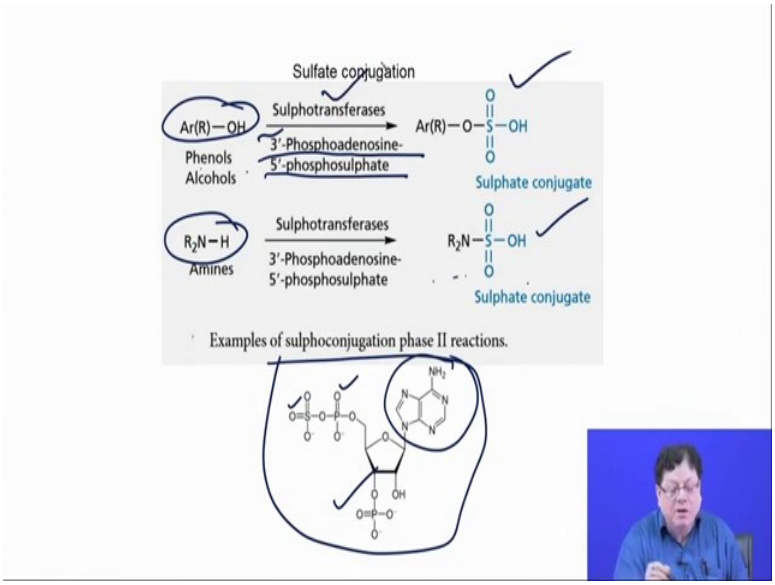


The other way is you can convert the OH or NH into sulphate. There are sulfotransferase enzymes. They actually form RO sulphate and then $R_2N$ sulphate i.e. sulphonic acid derivative. The agent for doing this sulfur transfer is this compound 5 -phosphosulfate and here it is adenine. So, the compound is 3' -phosphoadenosine-5'- phosphosulfate and the enzyme will be called as sulphotransferase.

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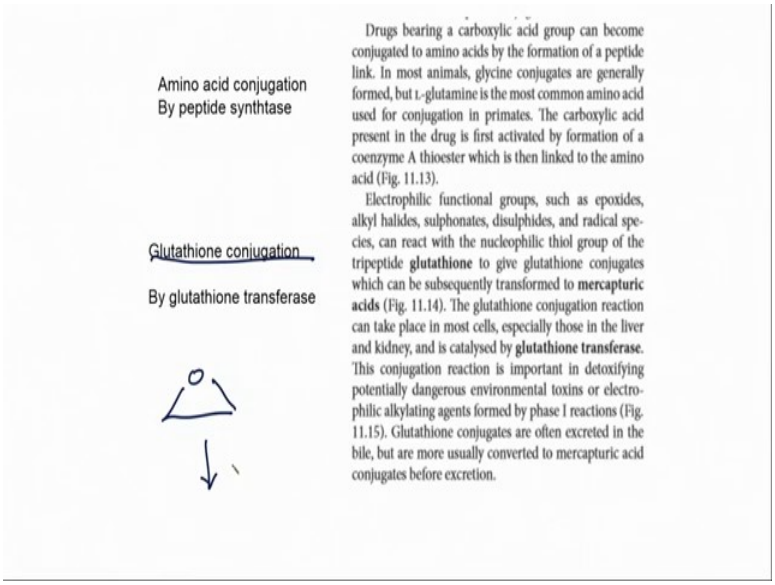

A third one is the glutathione conjugation. Glutathione is a tripeptide.

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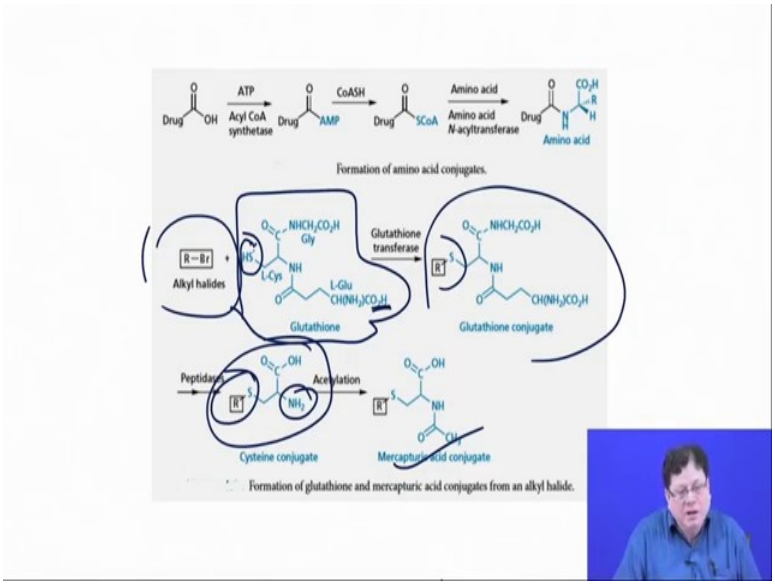


Glutathione contains L-glutamic acid linked to cysteine, linked to glycine. But remember in glutathione gamma carboxy of glutamic acid is attached to the cysteine and cysteine attached to the glycine.

It is an antioxidant due to presence of sulphur. It can be a very good bio-conjugating agent. If you have epoxide the sulfur being a nucleophile can open up the epoxide. If the drug is an alkyl halide sulfur will undergo alkylation but the whole thing is now soluble in water.

Further it can be hydrolyzed by peptidases into only the cysteine with attached to the drug. This is acetylated, this is called mercapturic acid. If you have a reactive functionality in drug then glutathione attacks the drug because it has got a powerful nucleophile SH. That attacks the drug and does alkylation or opening of epoxide but ultimately there is a carbon sulfur bond formation. The glutathione part can be degraded ultimately to the cysteine and that can be acylated and finally removed from the system. So, that is called metabolism.

We have gone through metabolism and excretion usually through the kidney. So, that is the part of the PK. Pharmacodynamics has already been covered. Pharmacodynamics means what the drug does to the body *i.e.* all the targets. Then we have said anti-cancer,

antibiotics, everything. In the next session, we will discuss about designing a drug again with all due regards to computational chemistry but ultimately there are certain other parameters which are very difficult to balance.

I said lipophilicity, hydrophobicity have to be balanced. But what is the value? How Lipinski arrived at those values? That log p should be less than 5. So, those are basically an entirely different area of medicinal chemistry and that is called quantitative structure activity relationship.