Computer Aided Drug Design
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Lecture – 13
Pgp efflux/Drug Likeness

Hello everyone, welcome to the course on computer aided drug design. Today, we will talk about the Pgp efflux and few more drug likeness property. Yesterday, after blood brain barrier penetration, I introduced what is Pgp, we will spend more time on this Pgp.

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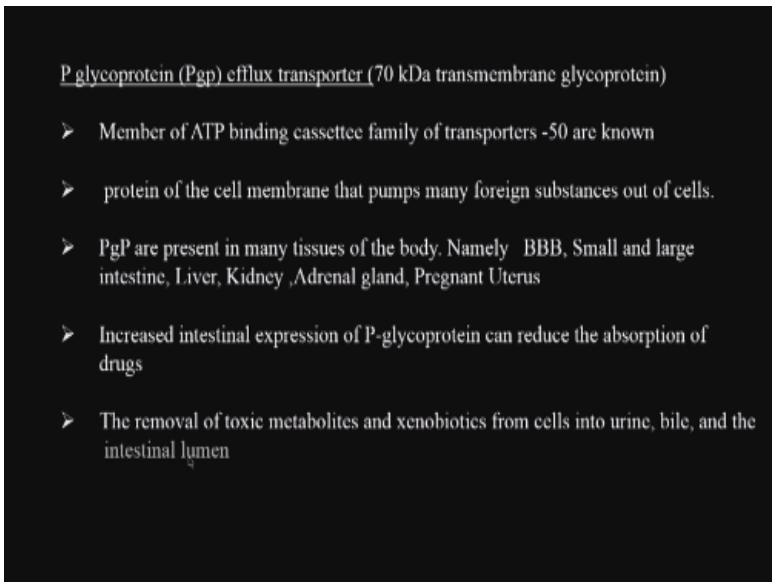

They are P glycoproteins, these are efflux transporters that means they throw drugs out from the cell inner, so that they maintain the concentrations of drugs at very low values, they are meant to do the job for toxins and toxic metabolites, these are transmembrane glycoprotein okay, they are 70 kilo Dalton in size, they are part of the ATP binding cassette family of transporters, there are 50 of them, large number of them as you can see.

These are cell membrane that pumps many foreign substances out of the cell, they are present in many tissues in fact more in some of the tissues like a BBB like I mentioned blood brain barrier has a lot of efflux transporters, small and large intestines, liver, kidney, adrenal gland, pregnant uterus, increased intestinal expression, so if you have a large amount of Pgp present in the intestine, so what it will do is; it will reduce the absorption of the drug through the GI to the plasma okay.

So, because it will try to efflux and remove the drug through the faeces, they are meant to remove toxic metabolites and xenobiotics in fact, that is their job to throw them into urine, bile and intestinal lumen okay that is the job but unfortunately they may even throw the drugs as well.

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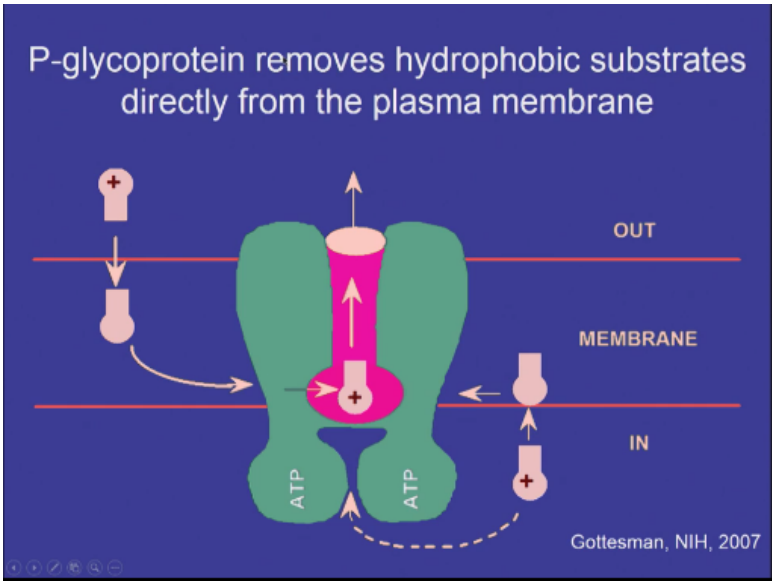

This picture was taken from this reference, so these removes hydrophobic substrates directly from the plasma membrane, so we have the plasma membrane just gets thrown out here okay, so the ATP plays a very important role here okay that is what P glycoprotein they are on the membrane bound as you can see here.

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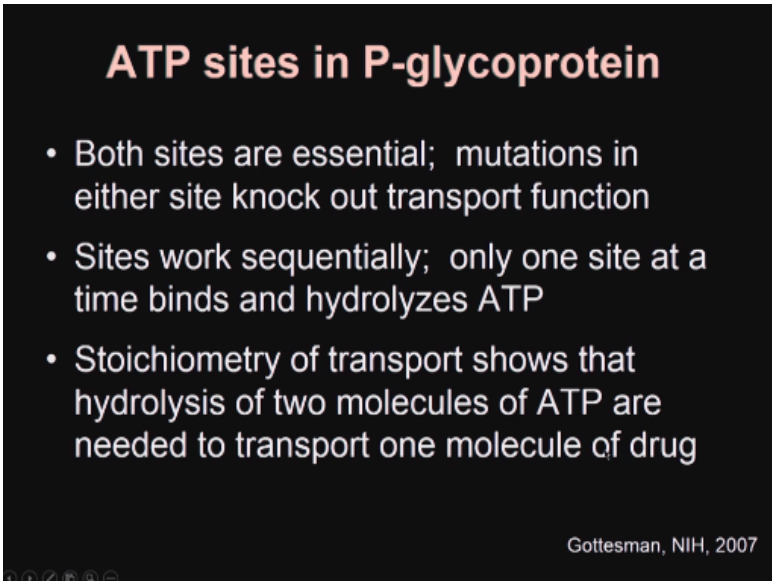

Both the sites are essential for this for, so for example it is got 2 different sites okay, so both are very important and mutations in either side knocks out transport function. The site works

sequentially only one side at a time binds and hydrolysis the ATP present here, so you require hydrolysis of 2 molecules of ATP to transport one molecule of this drug, so that is what it does.

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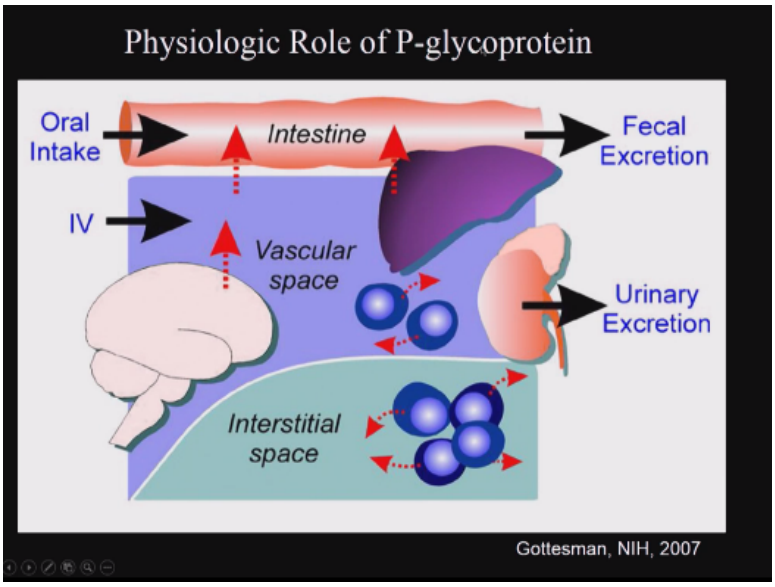

Throwing out drugs away and they are located at the membranes, so what is the physiologic role of this P glycoprotein? So, we have an oral intake, so we may have Pgp is here, so it binds toxic material and then it is excreted faecal excretion okay or if it is in the intravenous okay, then it binds and then it removes it through the urinary excretion. So, these are the 2 main jobs of P glycoproteins okay.

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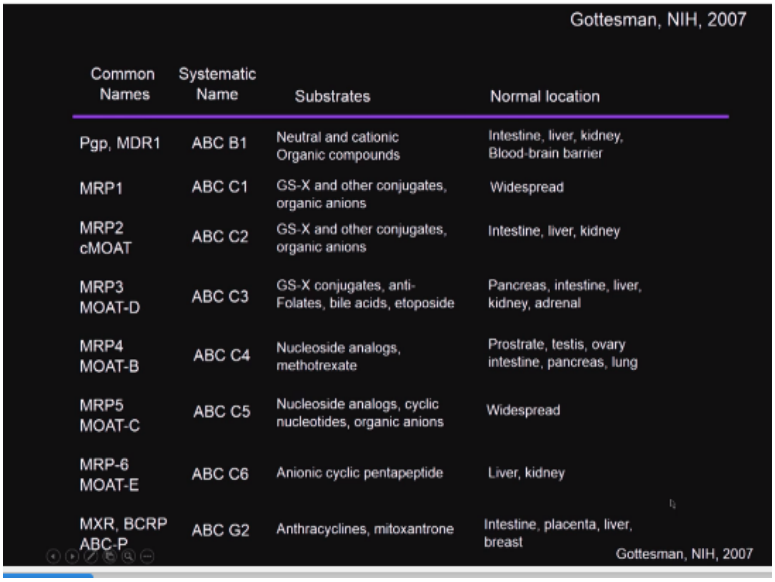

So, you have a very hydrophilic material, it may go through the urine, hydrophobic; it may go through the faecal and so on. So, there are large number of Pgp's, these are the common names; MDR1, MRP1, MRP2 and so on here as you can see, these are the systematic names. What are

the substrates for these Pgp is neutral and cationic organic compounds okay that is where your drugs may get caught.

Then we have MRP1 conjugates organic anions, conjugate organic anions, conjugates anti folates, bile acids, etoposide okay, nucleoside analogs, methotrexate, anionic cyclic pentapeptide, where are they found? For example, these Pgp's are found in intestine, liver, kidney, blood brain barrier this is where we are talking about; most of your drugs neutral and cationic organic compounds.

MRP 1; they are found widespread, these are found in intestine, liver, kidney and so on actually, if you look at MRP5, widespread and but they take in nucleoside analogs, cyclic nucleotides, organic anions and so on actually okay as you can see here where they are found, this is taken from this particular reference.

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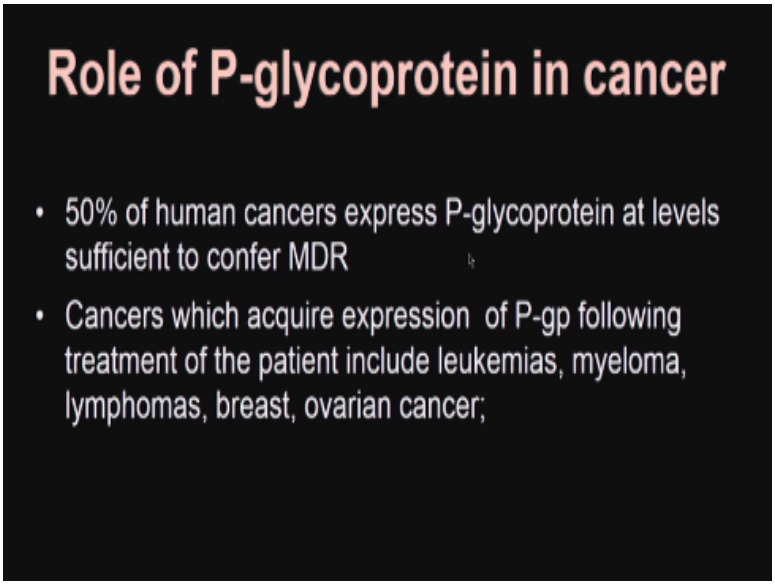

So, 50% of human cancers express large amount of P-glycoprotein, so what it does? It throws the drugs out, so the drugs do not act, so it becomes resistant okay, the cancer becomes resistance to drug therapy. Cancers which acquire expression of P-gp following treatment of the patient include leukemias that means after treatment initially, the treatment may appear to work but as they started expressing lot of P-gp's, then they start becoming a resistant to those drugs; cancers like leukemias, myeloma, lymphomas, breast cancer, ovarian cancer and so on the; it happens.

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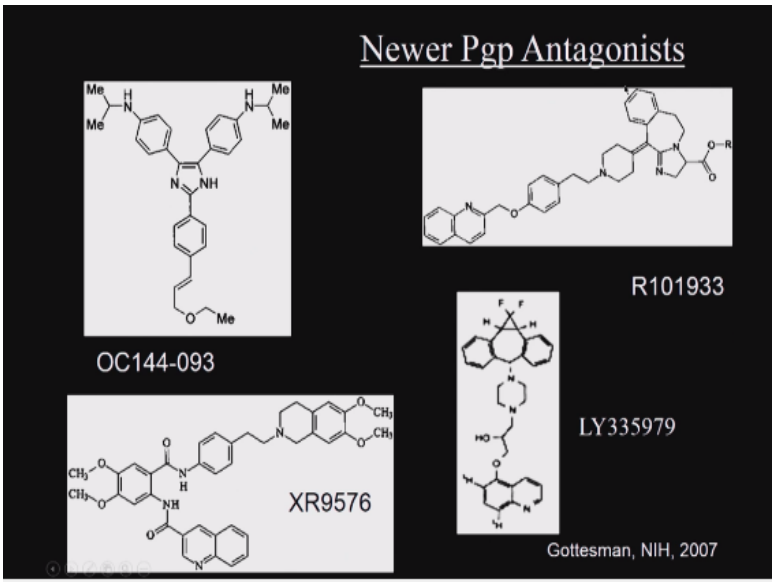

So, there are lot of Pgp antagonists that are currently in the either in clinical trials or about to enter the market, so these are some structures as you can see and these are new Pgp antagonists okay.

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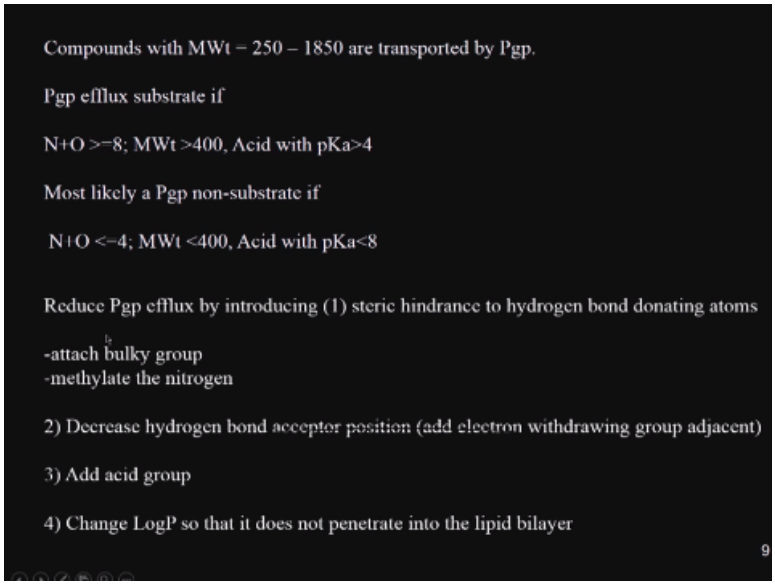

So, how do I design molecules if I want to avoid this Pgp? Compounds in molecular weight; 250 to 1850 are transported, so most of the drugs as you know can fall into this. So, if you have N + O > 8 or = 8 and molecular weight > 400, Acid with pKa > 4, then they can become a Pgp efflux that substrate that means, they can get thrown out by Pgp. If N + O < 4, molecular weight < 400, Acid with pKa < 8, okay then chances are they will not be Pgp substrate.

So, what it means is; I try to keep my nitrogen and oxygen quite low that means make it not too much hydrophilic in nature, make it to like by basically reduce Pgp efflux by introducing steric

hindrance to hydrogen donating atoms. So, if you how OH put OCH3, if you have NH put O, I mean NCH3, attach bulky groups, methylate the nitrogen. So, if you have NH, remove that H with CH3, decrease hydrogen bond acceptor position.

That means add electron withdrawing group adjacent, add acid groups, change log P, so that it does not penetrate into the lipid bilayer, so these are some strategies for reducing Pgp efflux okay.

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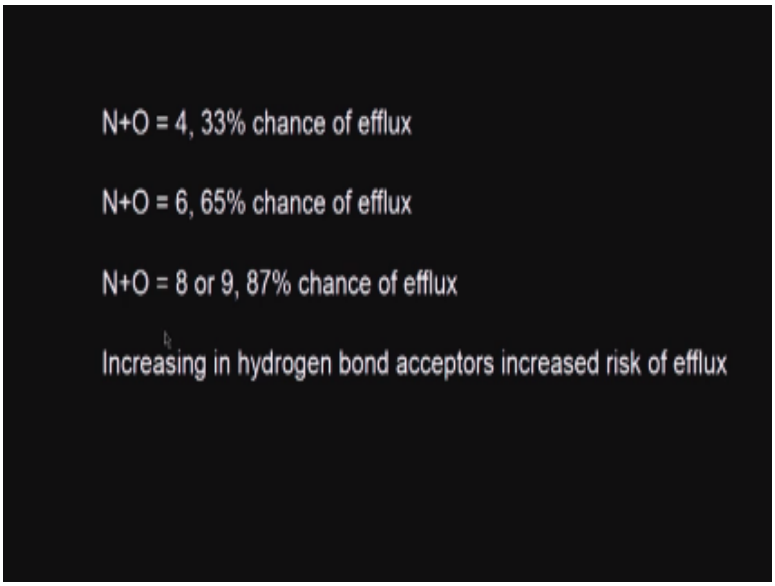

Nitrogen + oxygen; 4, 33% chance of efflux, nitrogen + oxygen; 6, 65%, nitrogen + oxygen; 8 or 9, 87%, so hydrogen bond acceptors could be a big culprit for Pgp, so if you have large number of hydrogen bond acceptors, the risk of efflux is very high. So, like I mentioned okay try to reduce that by putting in some bulky groups nearby okay.

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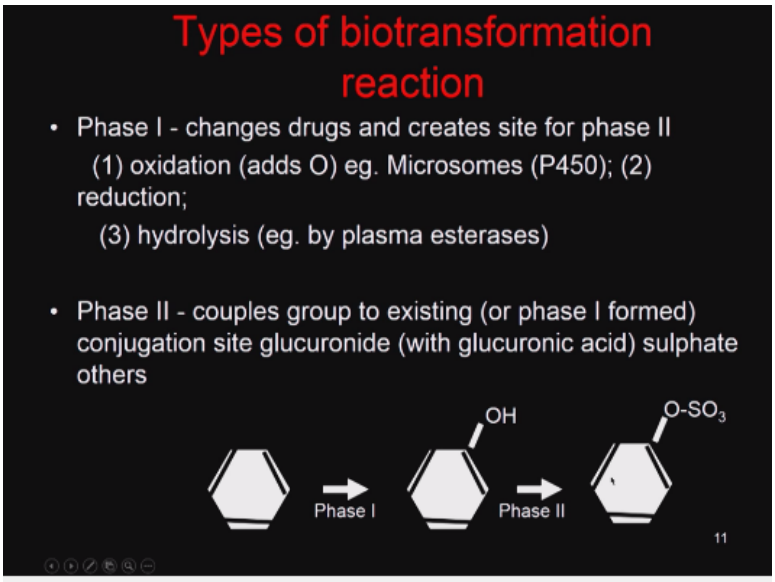

So, we talked about Pgp, let us again go back to the metabolisms in the ADME and let us look at how to overcome these bio transformations using structural modifications. Now, once more to revise, we have 2 types of metabolism; phase 1 bio transformation and phase 2. Phase 1 generally its oxidation that means add of O, okay reduction, hydrolysis okay by esterases enzymes.

Phase 2 is coupling these sides after phase 1 transformation by glucuronide with glucuronic acid or sulphate like in this picture, now you have an example of phase 1 hydroxylation and then you are putting a SO; sulphate has come here, so it is become more hydrophilic, so it can go through the urine okay.

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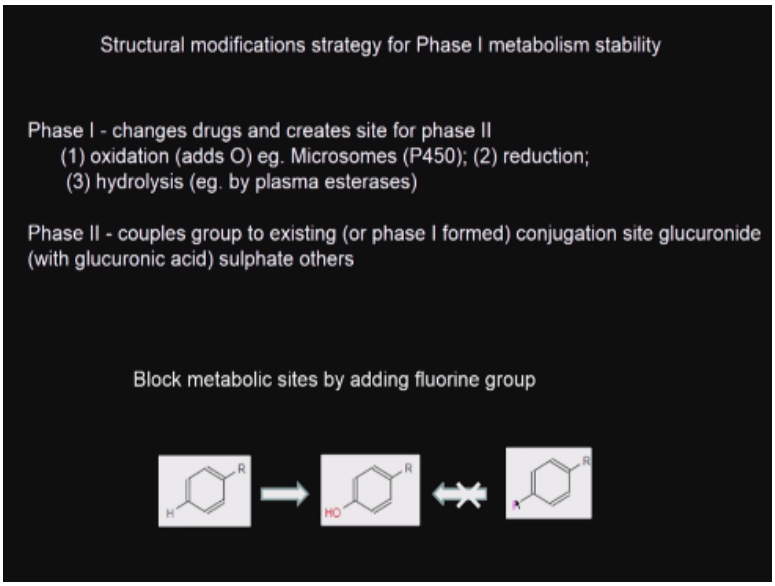

So, what can we do to avoid phase 1, so what does phase one do okay? Oxidation, reduction hydrolysis, phase 2; couples groups for existing, so you can block sides by adding fluorine group. For example, this can become OH okay then it is easy to put the phase 2; SO3, so what you do is; if you have a fluorine type of group, this type of reaction does not take place understand, so that is blocking metabolic sites by adding fluorine that is a good strategy.

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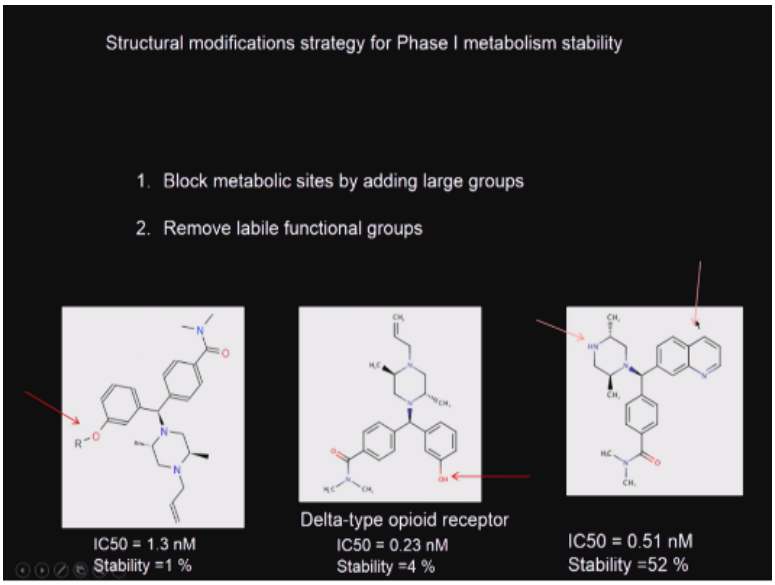

And in large groups, block metabolic sites by adding large groups, remove labile functional groups okay, so as you can see, IC50 is 1.3 nano molar stability is only 1% okay, look at this, this is a delta type opioid receptor, IC 50.3 nano molar stability is only 4%, so what has been done here; you put in some large groups here okay and then changes have been made here in all these places, so stability has increased to 52% okay.

So, metabolic sites you add large groups, you also prevent labile groups like this leaving okay by having large groups here and again you can see large groups here okay, so by doing that the stability of biotransformation has come up quite a lot, as you can see here okay.

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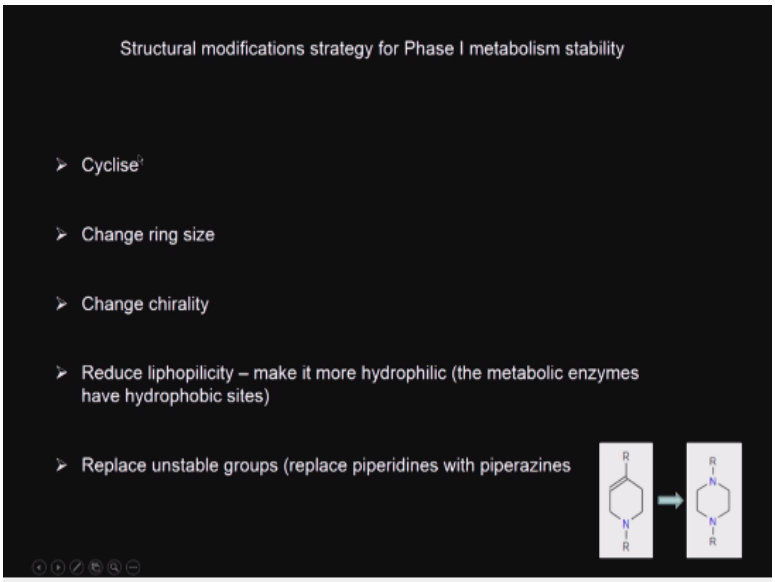

Because if you remove this and make a smaller group, the stability comes down here okay, okay, what are the things? Cyclise that means instead of linear make it cyclisation, change ring size, change chirality, reduce lipophilicity, make it more hydrophilic, the metabolic enzymes have hydrophobic sites, replace unstable groups. For example, if I replace the piperidine with piperazine okay, so then this becomes more stable than this.

So, what all can we do? We can cyclise thereby it prevents the metabolism, we can make the ring size different, change chirality make it more hydrophilic okay, okay so the metabolic enzymes have hydrophobic sites, so biotransformation, so you see in some cases we say make it more hydrophilic but if you want a drug to dissolve in the lipid layer and get absorbed you want it more hydrophobic.

If you want a Pgp efflux preventing substrate, we do not want too much of nitrogen and oxygen, so there are conflicting needs depending upon what we are looking at, so remember that drug discovery or sorry, drug design is not straightforward okay when you try to improve a parameter, other parameter can get to disturb okay. Replace unstable groups, so here we are trying to replace unstable groups here.

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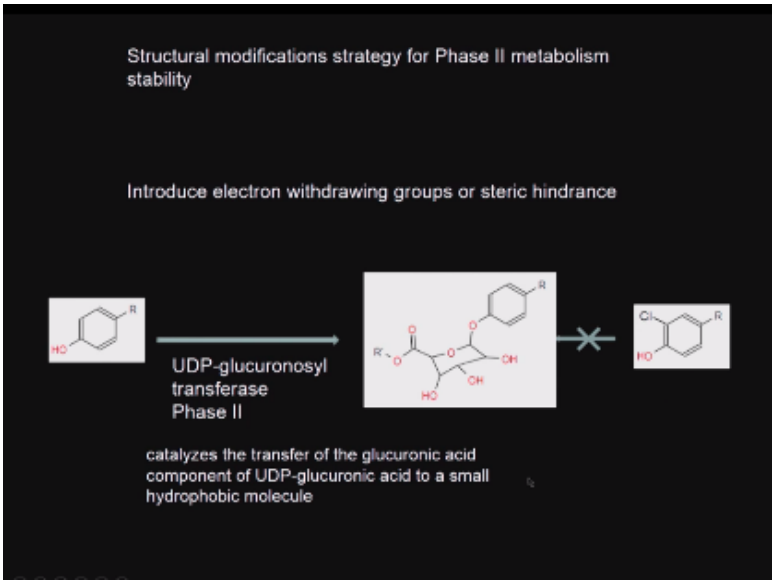

By replace as you can see piperidine with piperazine. Now, how do I prevent phase 2 metabolism? Introduce electron withdrawing groups or steric hindrance okay, for example you have this, in that phase 2 metabolism it can have like this, so it makes it into a hydrophobic molecule. Imagine when I put a chlorine this is an electron withdrawing group, this particular attachment does not happen okay.

Whereas, when you have this type of group, what happens? UDP glucuronosyl transferase phase 2, it catalyses the transfer of glucuronic acid component of UDP glucuronic acid to a small hydrophobic molecule, so you end up glucuronic acid coming here, whereas if I have an electron withdrawing group like CL what happens; this does not take place that means, phase 2 will not happen.

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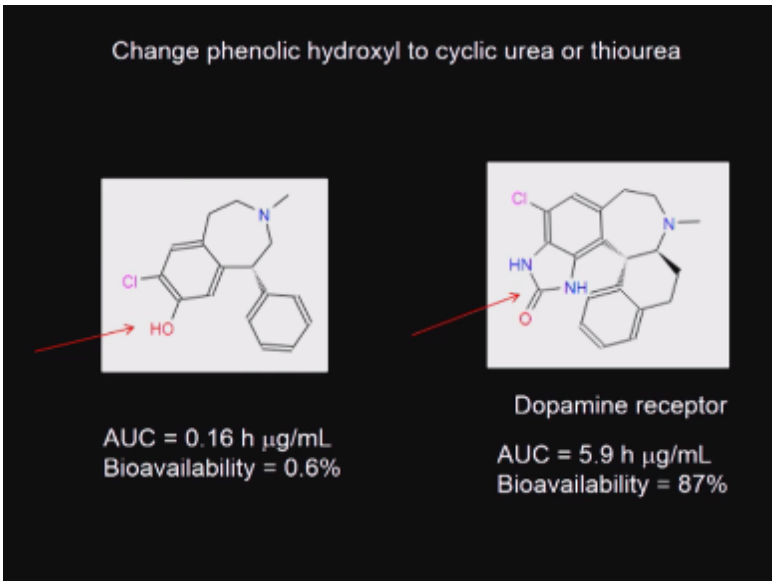

For example, you have a phenolic hydroxyl to cyclic urea okay that means, the cyclisation, so the bioavailability as you can see increases from 0.6 to 87%; big change, okay change phenolic hydroxyl to cyclic urea or thiourea that is what you are doing here, okay; cyclisation.

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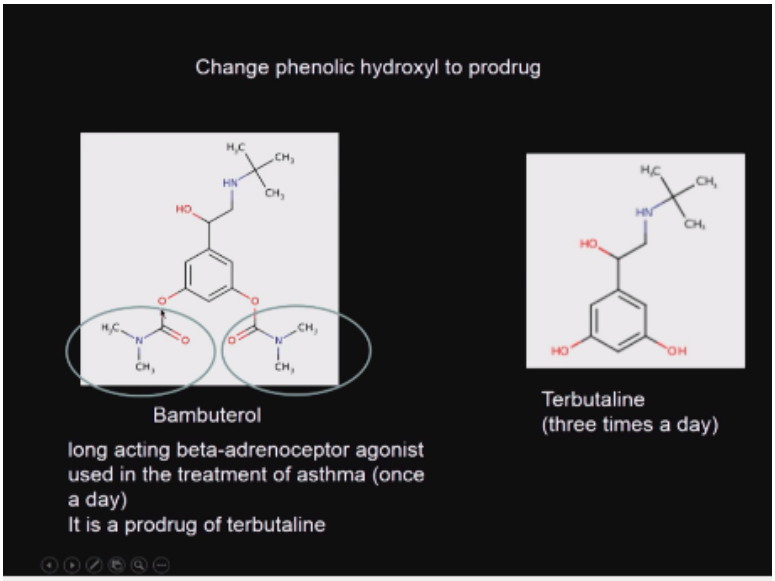

Change phenolic hydroxyl to prodrug, I did not talk about what is a prodrug. Prodrug; it is taken in orally, it is not a drug, it may contain another component which get cleaved in the plasma region because of the action of some esterase or lipase and one part will be the real drug which will start acting. For example, if you look at this particular drug; terbutaline it is given 3 times a day because it has got very poor stability.

Whereas, you make a prodrug out of it; Bambuterol okay, you put in these 2 groups as you can see these are ester bonds which can get cleaved with esterase enzyme, so these are called long acting beta adrenoceptor agonist used in the treatment of asthma, it is given only once a day okay, whereas this has to be given 3 times a day, whereas when I put in these 2 groups here and here, it acts as a prodrug, it is stable.

But it cleaves only in the presence of esterase type of N because you have the ester bond, so it is given only once a day, used in the treatment of asthma, you can see that.

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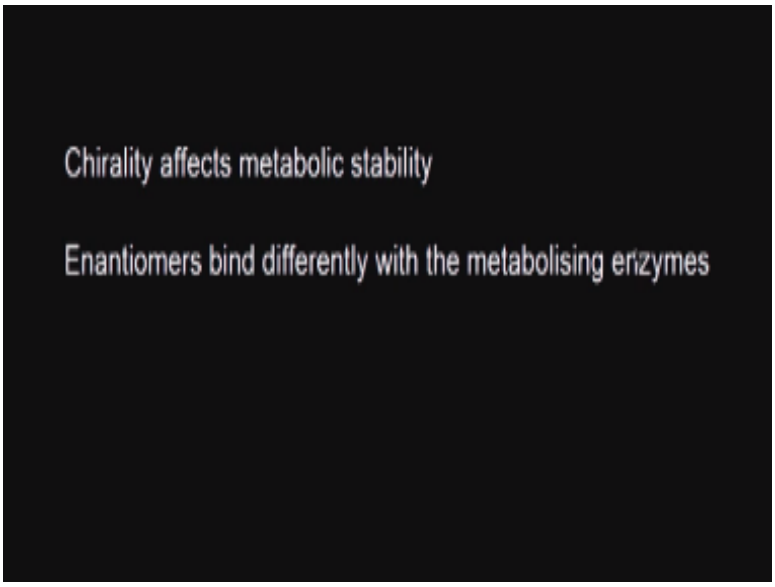

Chirality affects metabolic stability okay, Enantiomers bind differently with the metabolizing enzyme, right you have R Enantiomer and they may bind with the enzymes which are involved in the phase 1 and phase 2, so you may end up with either bio transformation happening or not happening okay.

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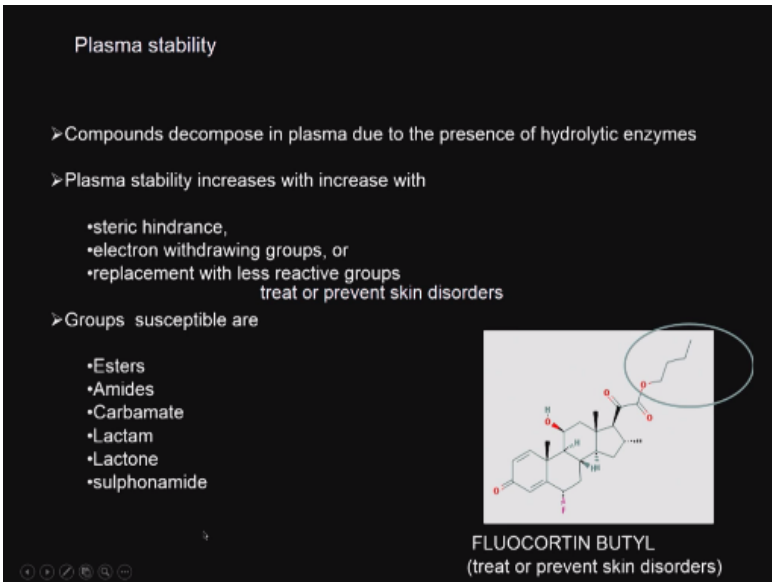

Next one is plasma stability; compounds decompose in plasma due to the presence of hydrolytic enzymes, plasma contains hydrolytic enzyme, so ester bonds can get degraded. Plasma stability increase with increase with steric hindrance so, you do not allow the hydrolysis taking place or you can put electron withdrawing groups or replacement with the less reactive groups okay.

For example, look at this compound; Fluocortin butyl, it is treat or prevent skin disorders okay, so you can have this bond breaking because of the hydrolysis, okay. Ester can get hydrolysed;

amides, carbamates, lactam, lactone, sulphonamide so if you have these groups, they have chances of poor plasma stability because there can be hydrolysis taking place here as you can see here, okay.

This bond can break, so what we can do; we can have big groups like here you can have electron withdrawing groups, so this cleavage does not happen, you can replace with less reactive groups, so that the cleavage does not happen but I remember this type of groups can have plasma instability esters, amides in this order esters extremely susceptible to hydraulic enzymes; amides, carbamates, lactam, lactone sulphonamide and so on actually.

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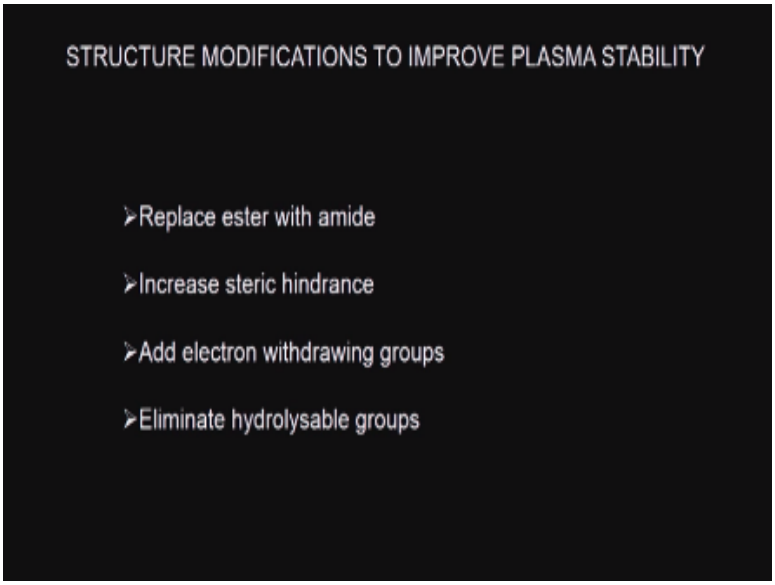

So, this is the strategy by which we can enhance plasma stability okay. So, what are various things we can do? Replace esters with amide because amides are less hydrolysable than esters, increase steric hindrance, add electron withdrawing groups, eliminate hydrolysable groups okay, these are the various strategies okay, we can do to improve plasma stability.

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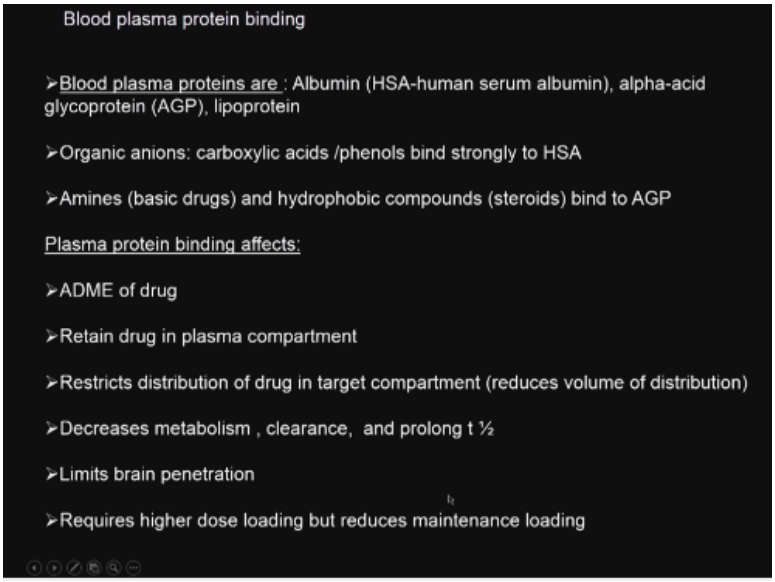

Of course, we also have these proteins in the blood plasma and compounds can bind to that thereby their amount, their concentration in the freely available form will be very less. So, we want to avoid that also, so blood plasma proteins are albumin like human serum albumin, alpha acid glycoprotein, lipoprotein these are the proteins found in the blood plasma. So, organic anion, carboxylic acids, phenols bind strongly to it.

So, if you have an acidic drug; highly acidic drug chances are; it can have a high plasma binding capability, so the amount of freely available drug in the blood will be reduced because of this. Amines; amines basic drugs and hydrophobic compounds like steroids, they bind to this glycoprotein okay whereas, some acid drugs can bind to albumin, so what does plasma protein binding effect of that.

It affects the ADME of the drug, it retains a drug in the plasma compartment, restricts distribution of drug in the target compartment that means because some of the drug is bound to the plasma, so the amount of drug available at the target site may be low okay, then decreases metabolism, clearance and it prolongs the half-life because some drug is already bound to the plasma and it may get slowly released over a long period of time.

So, you may have a fast drug available whichever is in the free form whereas, the drug that is bound to plasma will get slowly released and the concentration of the drug will keep persisting that is why it prolongs to the t half, it limits also the brain penetration because the amount of drug available is very less, you need higher dose loading but reduces maintenance loading.

Although you require higher doses because some drug has gone to the plasma bound the drug available for maintenance has gone down.

But you need require higher doses of loading okay, for example; one example you know MK 886, it is a drug, it is an acidic drug, it has been found very good for enzymes like prostaglandin e synthase mpges1, which is involved in arachidonic acid inflammatory but because of the high plasma binding, the drug has to be withdrawn, so it is an acidic drug.

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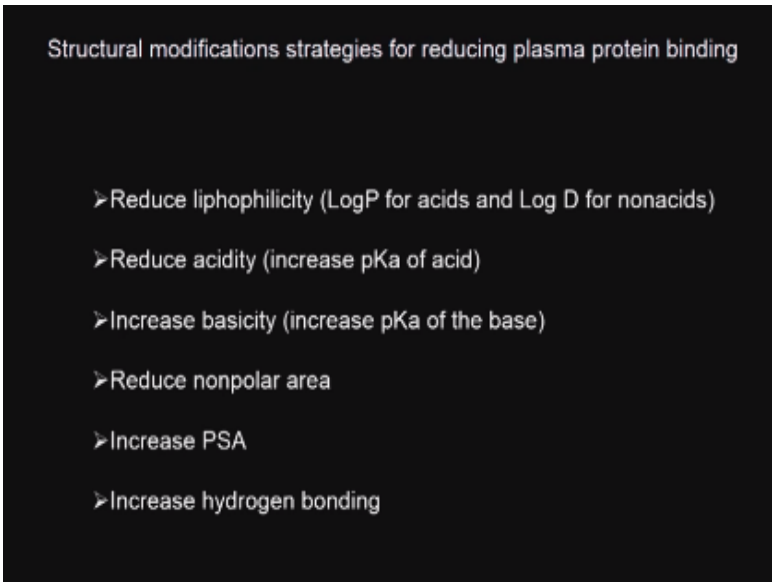

So, what are the structural modifications we can do? Reduce lipophilicity, so log P for acids and log D for nonacids, reduce acidity okay, increase pKa of acid, increase basicity, increase pKa of the base, reduce nonpolar groups, nonpolar area okay or nonpolar groups as we can see okay that is called reduce nonpolar groups, reduce nonpolar area, increase polar surface area that means increase polar surface area but reduce nonpolar surface area increase hydrogen bonding okay and so on.

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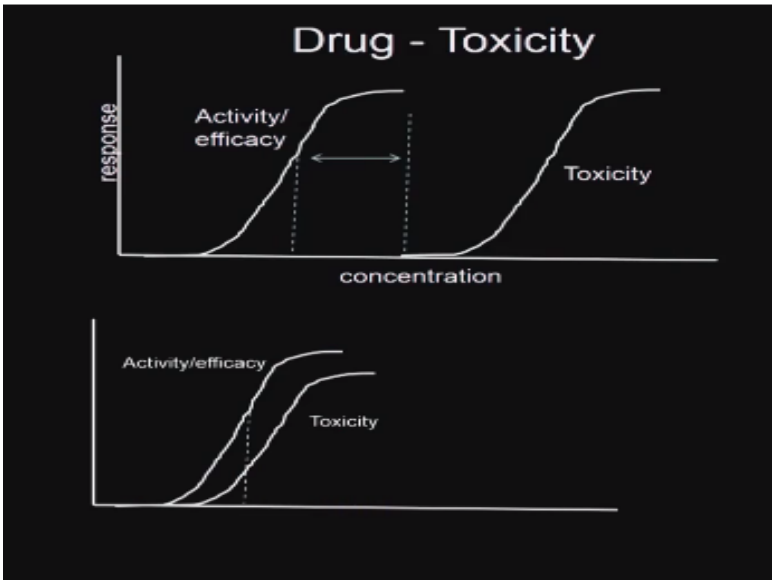

So, many different strategies one can follow to reduce the plasma protein binding effect okay. Toxicity is another important parameter, the drug toxicity which we need to consider, so when we say ADME now it has been extended to ADMET; absorption distribution metabolism excretion and toxicity. So, the concentration of the drug, the actual concentration that is used for treatment should be nowhere near its toxic limits.

The toxicity limits should be much farther okay, for example if this is the activity efficacy, okay for example, anti-bacterial if you take as I keep increasing concentration, it starts killing bacteria and it rises and reaches plateau but the toxic limit of that drug should be much far; much far okay, so I can operate in this concentration range but still it will not be toxic, whereas if this graph is closer, then this range becomes narrower.

So, I can operate with confidence because the toxic limits come only beyond this, you understand okay, the toxic limit is only beyond this, whereas this is the efficacy limit. So, ideally I should have a drug which shows a graph for the activity and which shows a graph for the toxicity. Whereas, if I have a graph for a drug which shows like this activity, like this toxicity, if I start using the drug in this concentration it can also have some toxicity as well okay which is not alone.

Whereas if I start using the drug at lower, the efficacy of the drug becomes lower and lower, so if I want to prevent toxicity, then efficacy is low. If I increase and improve efficacy then it also has toxicity, so ideally we should be having drugs like this, where the toxicity graph is far away from the activity graph, so this is the very ideal situation but many times you can have toxicity

coming in here like some of the chemotherapy drugs and so on which can be also toxic and it is active.

Because you have to give the drug at very high concentration, so we need to understand toxicity as well, the toxicity could be short term, long term, toxicity could be systemic, toxicity could be particular organ, different types of toxicities are there, we look at some of them in brief, okay.

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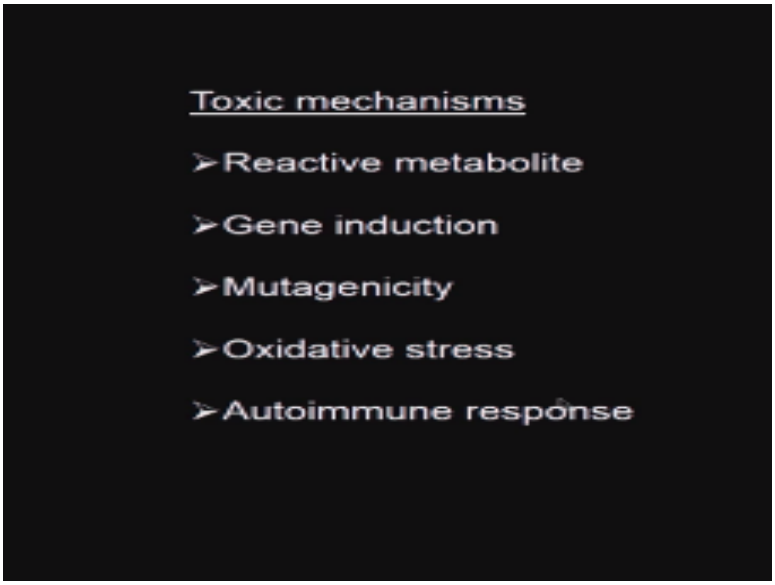

The reactive metabolite, so that could be toxic, gene induction, mutagenicity, oxidative stress, autoimmune response, so these are the various ways by which toxicity could be introduced because of the drug treatment.

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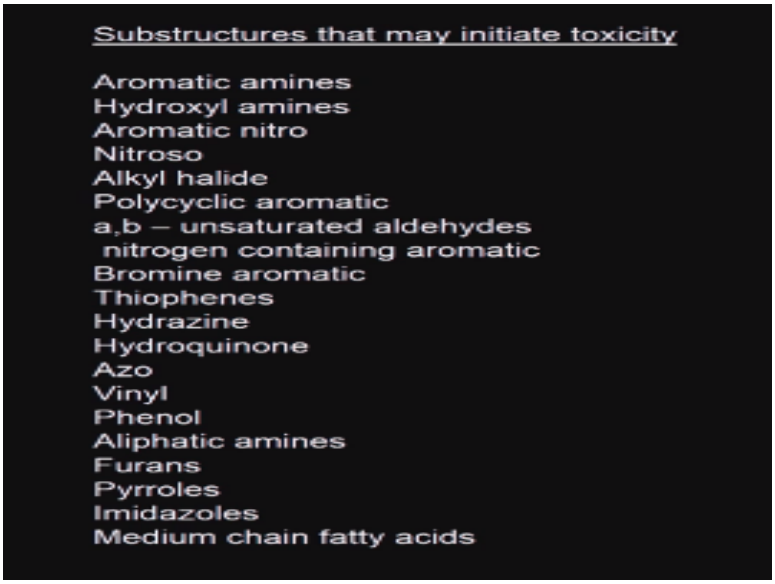

So, what are the substrate that may initiate toxicity, amines play a very important role; aromatic amines, hydroxylamines, nitro compound, so generally nitrogen based compounds can lead to toxicity, so you can see aromatic amines, hydroxylamine, aromatic nitro, nitroso okay then comes alkyl halides, polycyclic aromatic, alpha beta unsaturated aldehydes, nitrogen containing aromatic, bromine aromatic, Thiophenes, hydrazine, hydroquinone, azo, vinyl, phenolic, aliphatic amines, furans, pyrroles, imidazoles, medium chain fatty acids, so it comes like this.

So, these type of structures as you can see many of these structures can be toxic because some of the metabolites could end up being toxic okay that is a big problem okay and the toxicity can be of different in nature.

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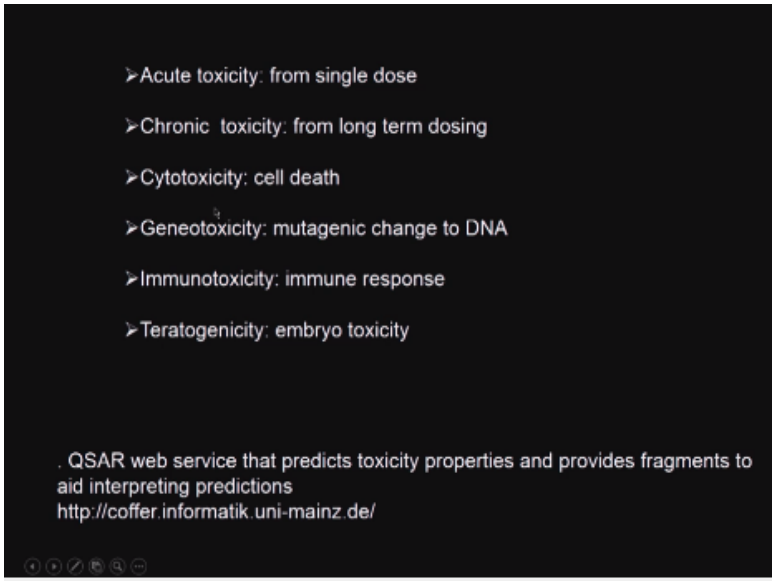

Acute toxicity from single dose, I give one dose and it is toxic, so that is called acute, chronic is from long term dosing that means the drug is given over a period of a few weeks, few months, few years and so on. Cytotoxicity; so the drug kills cells, genotoxicity; there is some mutagenic change to the DNA, immuno toxicity; the immune response is affected okay, immune response is compromised that is called immunotoxicity.

Teratogenicity; embryo toxicity, okay so different types of toxicities, so one needs to study all these if one is designing drugs because it might not be a single dose, it could be long term dose, cell death; lot of mutagenic changes to the DNA can happen it may affect immune response or some of these toxicity you may be able to find out on long term studies, some of these could be the short term.

And some of them needs to be studied in very specialized condition to look specifically looking at embryo and so on actually, in factor there is a website http coffer informatik; you look at this, QSAR web service that predicts toxicity properties and provides fragments to aid interpreting predictions okay, so you give a structure, it may able to predict the toxicity of that compound and also look at the fragments and also make comparison and certain conclusions.

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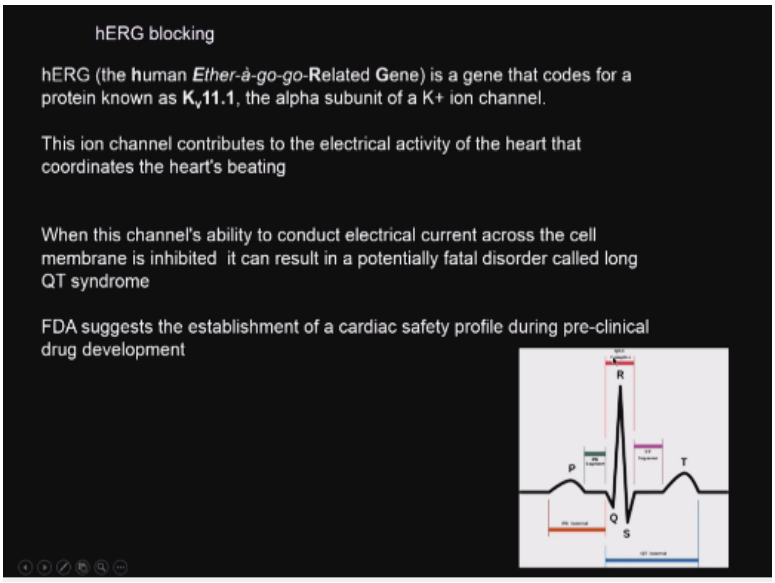

And so there are many websites and this is quite a good website, when we have time we will look at this as well okay. So, we looked at toxicity okay that is another aspect, now let us look at another important aspect that is called hERG, the human Ether a- go- go related gene okay, Ether a- go- go related gene, this is a gene that codes for a protein known as Kv 11.1, the alpha subunit of a potassium ion channel.

This ion channel contributes to the electrical activity of the heart that coordinates the heart beating, so this particular; we have to look at this particular gene and see whether the drug is going to affect this because if so, it may affect the heart beating okay. When this channel's ability to conduct electrical current across the cell membrane is inhibited, it can result in a potentially fatal disorder called the long QT syndrome.

So, as you know if you remember how we have typical ECG looks like, so we have the initial P okay and then we have this is called the PR then goes down Q then R is high and then again it goes down S, then this is called T okay, so sometimes this gets extended okay that is called the extended QT syndrome okay, long QT syndrome okay. So, because the potassium ion channel gets blocked because of your drug and this can lead to cardiac problem.

So, FDA suggests the establishment effect cardiac safety profile during preclinical drug development, so you need to study whether your drug affects this pattern, especially the Q and T part of it, QT this is called the QT interval okay, this is called the PR segment, this is called the ST segment and so whether there is a drug affects this is what they need to see. So, we need to look at this.

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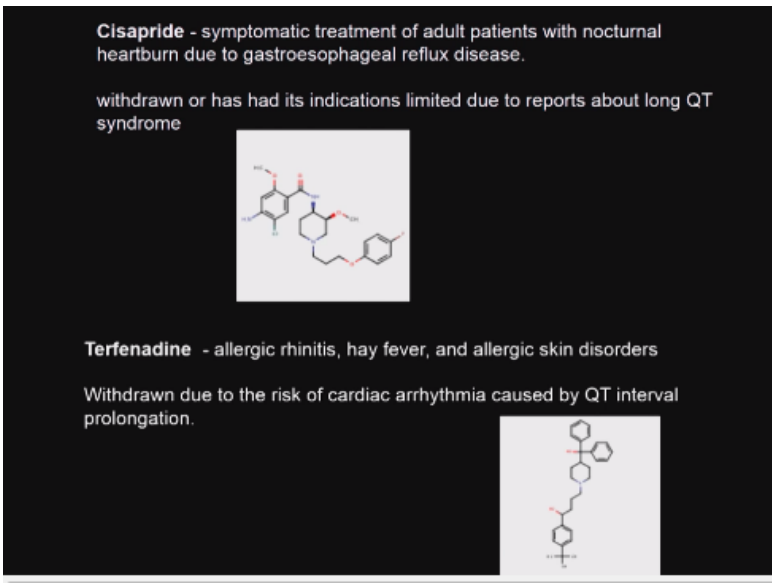

So, generally drugs are checked and see whether it affects this particular potassium channel and hence the QT part of the time. Look at this drug; Cisapride; symptomatic treatment of adult patients with nocturnal heartburn due to gastroesophageal reflux disease okay, so as you can see sometimes you can have an acid reflux coming in, then it can lead to heartburn, this drug was withdrawn or had its indication limited due to the report about long QT syndrome.

So, this drug along elongates the QT time period okay, another drug; terfenadine, this is allergic rhinitis, hay fever, allergic skin disorders, this was withdrawn due to the risk of cardiac arrhythmia caused by QT interval prolongation again it is because of long QT interval. So, these drugs directly affect the cardiac function okay, so they were withdrawn, so you need to understand whether the potassium channel ion channel gets affected because of blocking of this hERG gene which in turn affects the cardiac rhythm especially the QT portion of the ECG okay.

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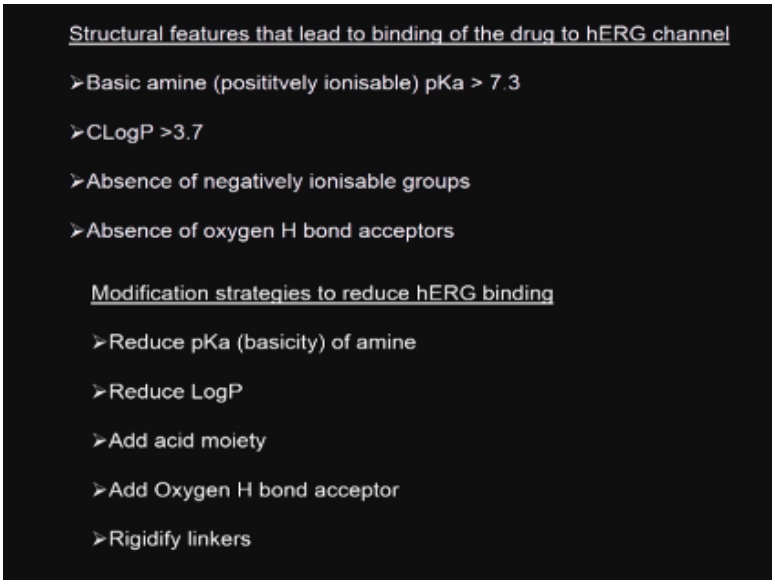

So, what are the structural features that lead to binding of the drug to hERG? Basic amines, positively ionisable pKa > 7.3, log P > 3.7, absence of negatively ionisable groups, there are no negative ionisable, absence of oxygen hydrogen bond acceptors, so these can lead to; so what modifications we can do to prevent hERG binding? Reduce pKa okay sorry; reduce pKa, basicity of amine okay.

So, there is a mistake here, reduce; reduce pKa of amine, make it basic; reduce log P okay because add acid moiety, add oxygen hydrogen bond acceptor because it is absence then chances are it could be binding to hERG channel, rigidify linkers that means reduce flexibility of these molecules and so by doing that we can prevent this hERG binding and hence we can prevent the disturbance caused to the potassium channel.

And hence the cardiovascular arrhythmia extended QT syndrome okay, so we will continue more on this drug likeness in the next class as well, okay. Thank you very much for your time.