Computer Aided Drug Design
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Lecture – 12
Drug - BBB

Hello everyone, welcome to the course on computer aided drug design. Today, we will talk about another important factor that is called the blood brain barrier okay, we want some drugs to penetrate this blood brain barrier drugs which treats certain central nervous system diseases, drugs which DEA treats bacterial infection in the brain and so on whereas, we do not want other drugs to penetrate this blood brain barrier and disturb okay.

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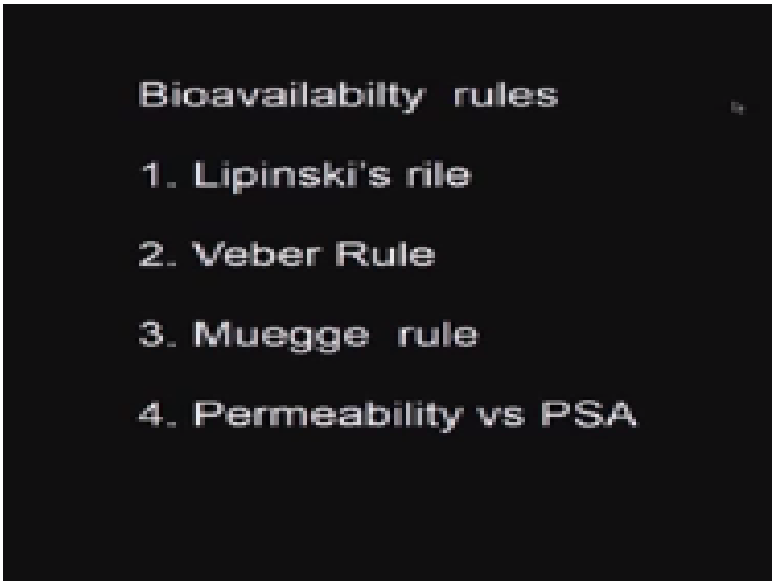

So, that is a very important concept which we are going to learn before that in the previous class we looked at various bioavailability rules in Lipinski's rule of 5 it is called, Veber rule, Muegge rule, permeability verses polar surface area, so these rules are very important when you are looking at oral bioavailability of molecules okay, we many softwares do the calculations and check whether these rules are satisfied.

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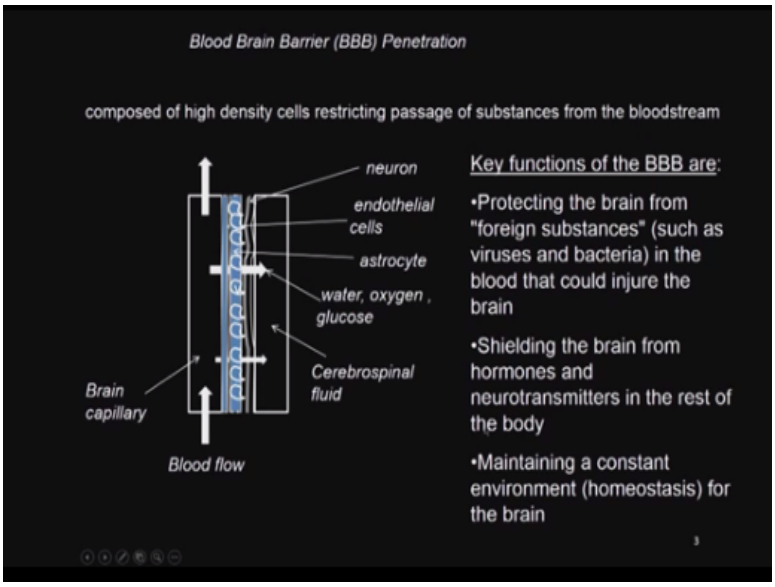

As we go along we are going to see some more rules as well okay, so what is this blood brain barrier okay and how important this penetration is? So, this is composed of high density cells okay restricting passage of substances from the blood stream. So, we have the blood flow here okay, then we have the brain cerebrospinal fluid CBF it is called, so some things pass through; nutrients may be, oxygen may be, okay.

But we do not want many things to pass through and disturb, so this is the neuron, these are the endothelial cells is called astrocyte, so it allows of course as I said nutrients, oxygen, water, so basically it protects the brain from foreign substances such as viruses, bacteria that may be present in the blood okay, it may; it prevent large molecules to pass through, so it shields the brain from hormones and neurotransmitters maintaining a constant environment and is called homeostasis, okay; homeostasis; constant environment.

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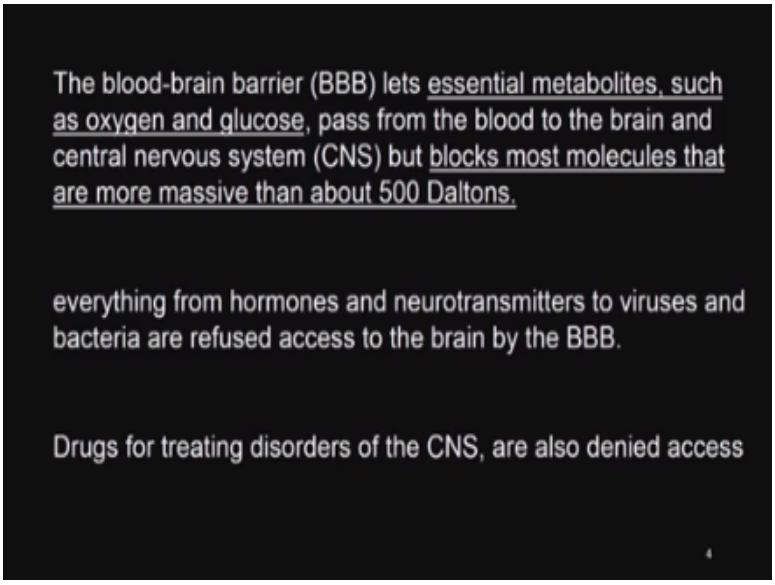

So, it allows essential metabolites, oxygen and glucose okay but blocks most molecules that are bigger 500 Daltons or more actually like hormones, neurotransmitters, viruses, bacteria are prevented from getting through. So, of course we want drugs that treat the central nervous system to pass through, so we need to design drugs, so that the structural features allow it to pass through the BBB.

So, we have an interesting situation, we do not want normal drugs to pass through the BBB, whereas if you are designing drugs for CNS or brain, we want those drugs to pass through, okay.

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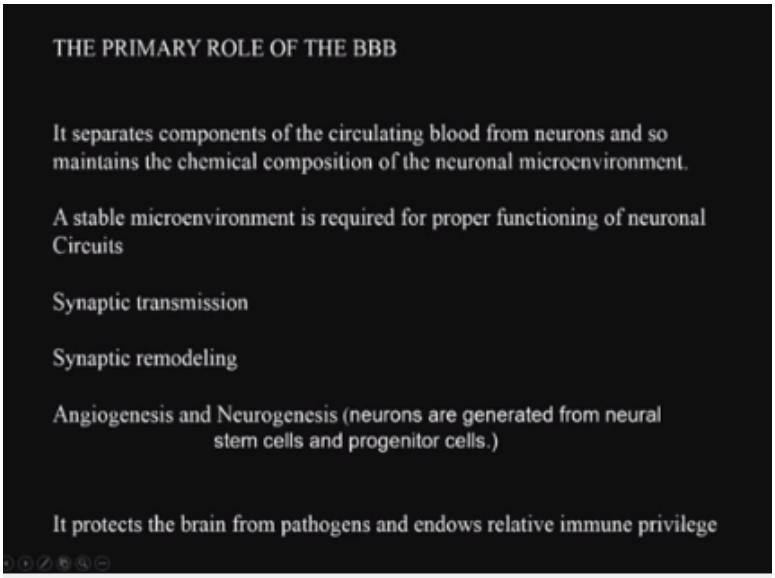

So, what are the primary roles of this BBB? Like I said it separates components of the circulating blood from neurons and so maintains the chemical composition of the neuronal

micro environment okay. This is a stable environment that is needed for the proper function of these neuronal circuits okay, so it is almost like electronic circuits inside, so as you know you need to maintain all the conditions constant.

There is synaptic transmission taking place, synaptic remodelling; it gets changed because of the environment and because of the exposure to the external environment and then angiogenesis and neurogenesis; there is neurogenesis means neurons are generated from neural stem cells and progenitor cells, so these things are happening inside the brain and so BBB has to take care of for this.

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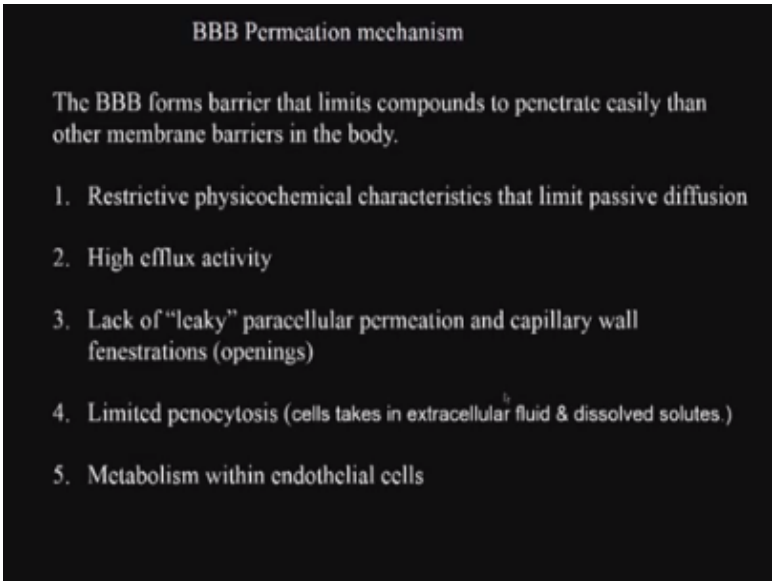

It protects the brain from pathogens and endures relatively immune privilege, okay. So, what is the mechanism? It forms a barrier that limits compounds to penetrate easily okay, this barrier is very tight because some many barriers; membrane barriers may have like holes they are not loosely; they are loosely joined, so compounds can pass through these loose joint, whereas blood brain barrier is very tight.

So, there are no gaps, restrictive physicochemical characteristics that limit passive diffusion, so it does not allow passive diffusion to happen, high efflux activity, so there are a lot of Pgp type efflux systems which throws whatever compounds that enter into okay it has got very high set of efflux pumps inside, lack of leaky; like I said lack of leaky paracellular permeation and capillary wall fenestration; openings.

That means, whereas in other membrane you may find a lot of capillary wall fenestrations but here you will not find any capillary wall fenestrations and especially in the BBB region okay, so it prevents this leaky permeation. Limited penocytosis okay, what is penocytosis? Cells take in extracellular fluid and dissolved solutes, okay that is called penocytosis, it is like gobbling up, eating up.

And so it does not have much of these penocytosis happening, so material cannot flow through okay that is like eating up or gobbling up, it is not the permeation type of thing and there is metabolism within endothelial cells okay, so compounds can get metabolized and you need uptake transporters that means there has to be transporters which pulls; takes these material inside.

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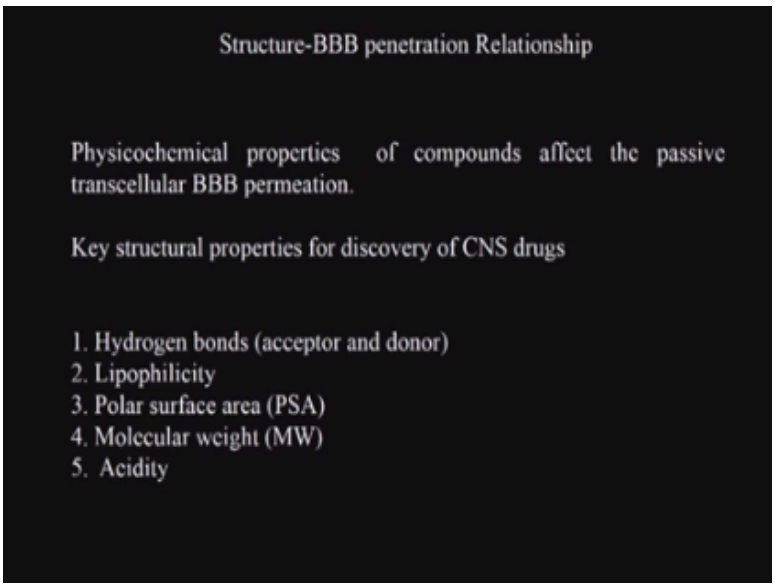

So, these are the mechanisms by which permeation in BBV takes place okay. So, what is the relationship between the chemical structure and BBB penetration? The chemical properties affect the passive transcellular BBB permeation. So, hydrogen bonds; acceptors, donors they play a very important role. Lipophlicity, because we have cellular membrane, so molecules have to be more lipophilic for it to penetrate rather than hydrophilic.

Polar surface area has to be limited, molecular weight cannot be very large, which large it will not go, acidity or basicity; the pKa value plays a very important role. I will show you that basic molecules can penetrate through, whereas not the acidic ones, okay.

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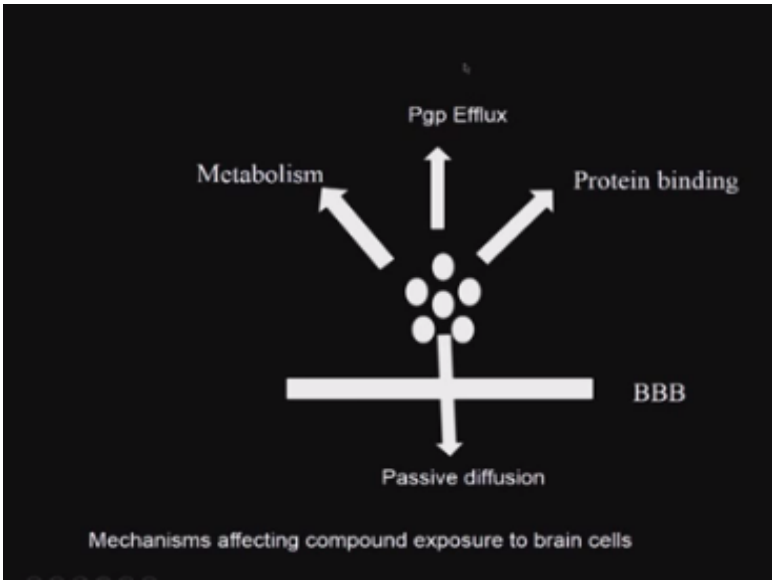

Okay, so how does it affect; how does the mechanism affecting compounds exposure? Okay, so we have passive diffusion taking place here, Pgp efflux that is throws it out then we have protein binding, so again the compounds get neutralized or metabolism, you could have lot of metabolism taking place, so the components get degraded, so these are preventive steps okay. The efflux is very high; binding to protein is also very high.

Metabolized products, small products which becomes harmless okay and generally it is; you have the passive diffusion like I mentioned in the previous case you do not have this okay, penocytosis taking place here okay.

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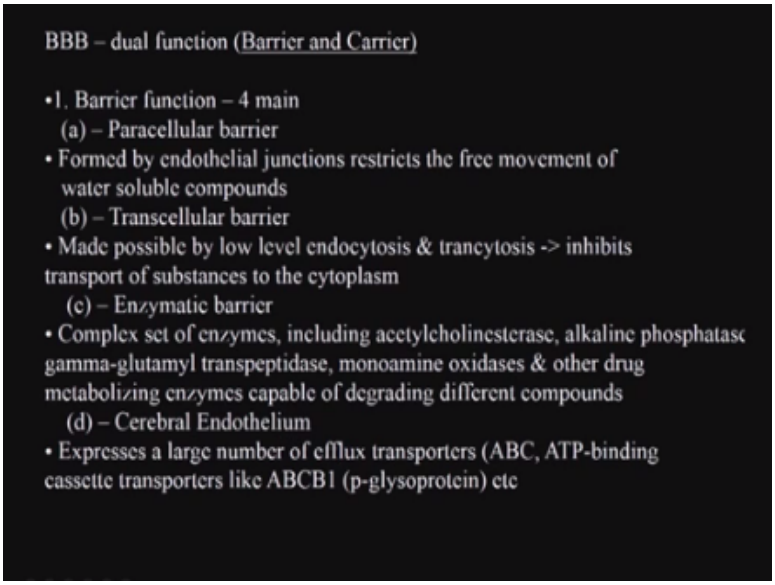

It does many things okay the BBB barrier and as well as the carrier, so what are these barrier functions? 4 main paracellular barrier; this is formed by endothelial junctions, so it restricts the

free movement of water soluble compounds. Water soluble means hydrophilic compound then you have the transcellular barrier because you have very low level of endocytosis that is gobbling up and transcytosis though so it inhibits transport of substances to the cytoplasm.

You have the enzymatic barrier; there are a lot of enzymes including acetylcholineesterase, alkaline phosphatase, gamma glutamyl transpeptidase, monoamine oxidase and other drug metabolizing enzymes, so they keep on degrading okay like I showed you here you know metabolism that is the enzymatic barrier. Cerebral endothelium expresses a large number of efflux transporters okay.

Here, the large number of transporters; ABC transporters, ATP binding cassette transporters like ABCB1 and so on, p glycoprotein. So, 4 different barriers are there, paracellular that means it formed endothelial junctions, it restricts water soluble compounds. Transcellular barrier, low level of endocytosis and trancutosis, enzymatic barrier; there are a lot of enzymes which degrade material inside, coming in.

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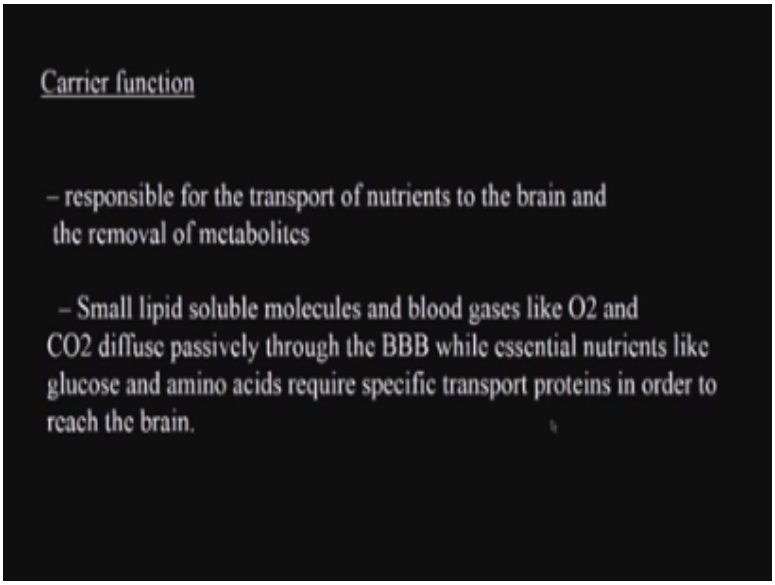

Cerebral endothelium; there are a lot of efflux transporters, so these are the various barriers okay. Now, carrier; these are responsible for carrying material from outside into inside like nutrients to the brain and so the removal of metabolites okay, these are small lipid soluble molecules and blood gases like O2 and CO2 diffuse passively through the BBB while essential nutrients like glucose and amino acids required specific transport proteins in order to reach the brain.

So, we have proteins which are very specific for transporting the nutrients, the amino acids inside whereas gases like CO2, O2 diffuse in a very passive manner, this is the carrier. So, 2 different functions of the BBB, the barriers; a lot of barriers here and the carrier we have carriers responsible for the nutrients through it and removal of metabolites and the gases passively diffuse into this BBB okay.

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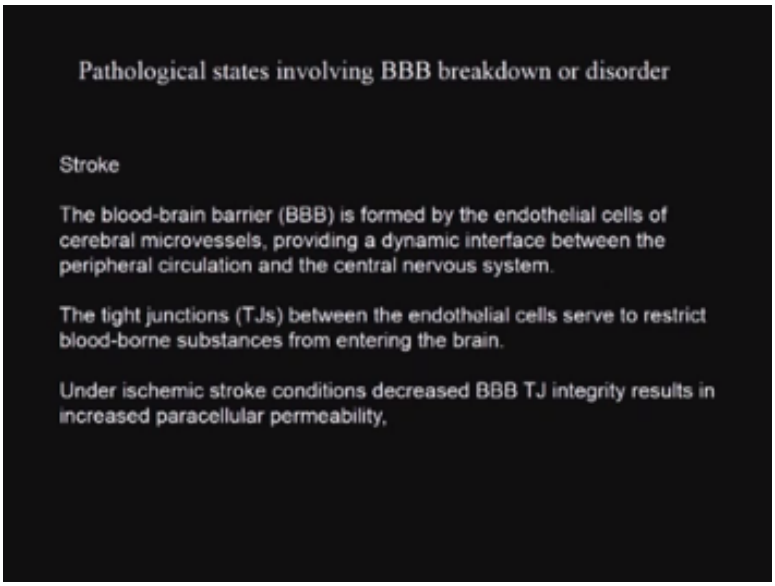

So, what are the pathological states involving BBB breakdown? So, BBB can also break down, so if it breaks down what happens; people may start having a lot of side effects, if they are given even normal drugs, even maybe inflammatory drugs or cardiovascular drugs because it is broken down. Stroke; for example, so the blood brain barrier is found by endothelial cells of cerebral microvessels provides a dynamic interface between the peripheral circulation and the central nervous system.

So, it has got a lot of tight junctions, they are called TJ between the endothelial cells serve to restrict, blood borne substances. Under ischemic stroke, it decreases this TJ integrity that means, there could be a lot of paracellular permeability, though the tight junctions get okay loose, so lot of gaps are produced and it improves the paracellular permeability okay, stroke is one.

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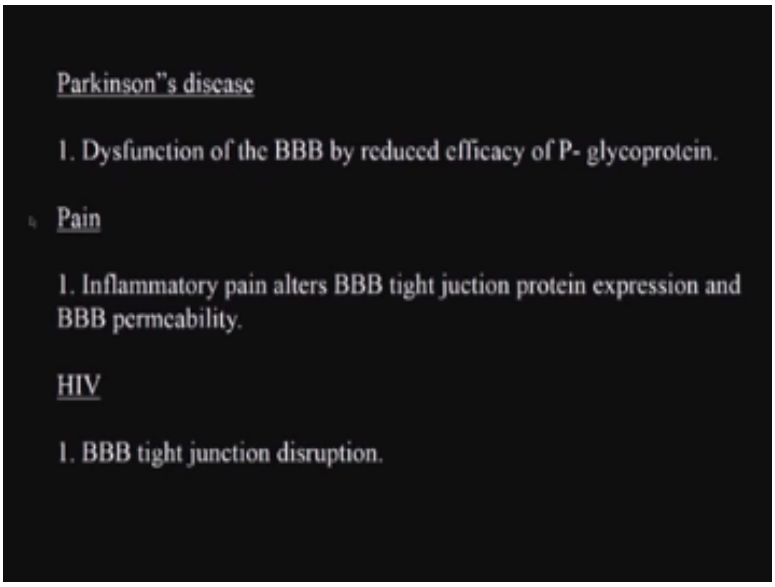

Parkinson disease; Dysfunction of the BBB by reduced efficacy of P glycoprotein that means the efflux pumps do not work very efficiently, so whatever material come inside do not get thrown out. Pain; so inflammatory pain alters BBB tight junction protein expression okay, it affects the protein expressions and it also affects the BBB permeability. HIV, again it affects the tight junction disruption.

So, there could be; because the tight junction has been compromised, you may have paracellular permeability, so all these diseases can have effect on the BBB and hence like I said instead of BBB preventing drugs, compounds, neurotransmitters from the body into they may start allowing them generally, the tight junctions get compromised here okay.

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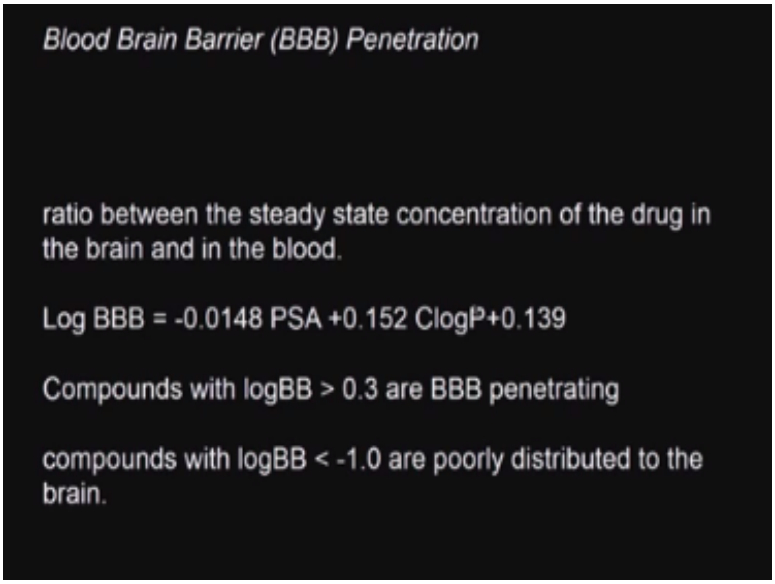

So, there are rules by which we can think of designing some molecules, which can be BBB penetrating or not. For example, ratio between the steady state concentration of the drug in the brain and in the blood that is log BBB is given by this relationship PSA is the polar surface area, log P is the hydrophobic, hydrophilic balance, so if you calculate this log BBB using this equation if it is > 0.3 okay, then we can say they are penetrating.

If they are < -1, they are poorly distributed that means <1 means, it could be very hydrophilic right, so they do not enter the BBB okay, whereas if it is > 0.3, they enter. So, polar surface area plays a role, log P plays a role, polar surface area is higher means, the molecules are hydrophilic in nature, log P is higher means the molecules are hydrophobic in nature, so you can see those 2 play important role in the BBB penetration.

This term BBB is the ratio of the concentration of the drug in the brain and in the blood, this is one data; one rule which says whether the molecule is permeating through the BBB or not, okay.

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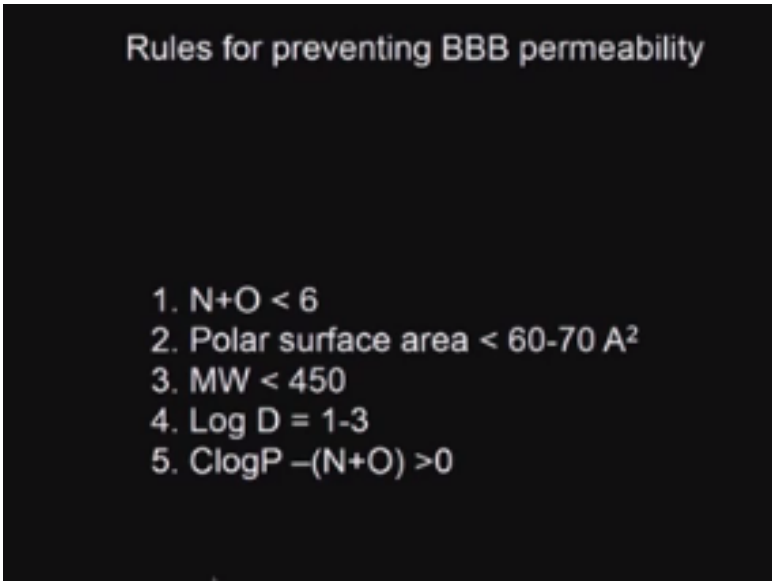

Other rules are number of nitrogen + oxygen should be < 6 to prevent a BBB, polar surface area; 60 to 70, molecular weight < 450, log D; 1 to 3, okay log P - N + 0 and N + oxygen should be > 0, so these are some other parameters, which also tells you whether a molecule can be BBB permeable or not BBB permeable okay.

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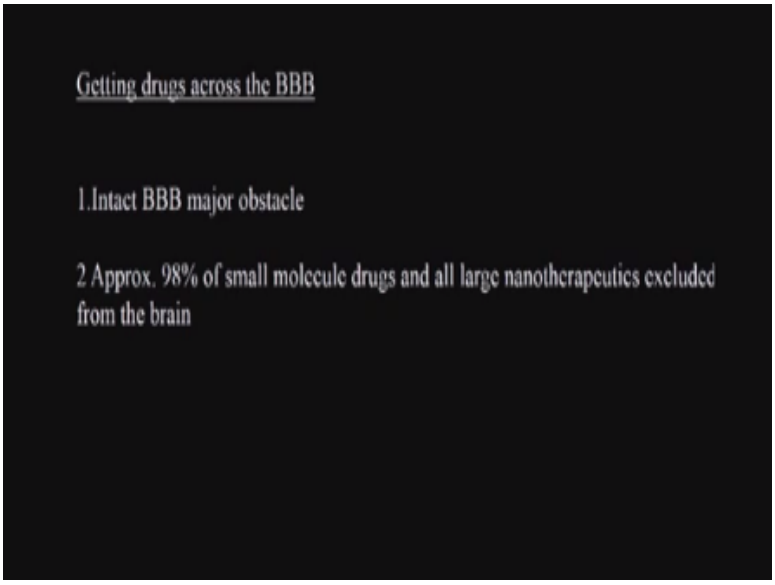

So, getting drugs across BBB, like I said that you need drugs, if you are treating CNS; if you are treating infections in the brain or if you are treating a cardiac brain disorder, so we need to have intact BBBS that is a major obstacle. So, 98% of small molecule drugs and large nano therapeutics excluded from the BBB like molecular weight, if it is very, very large of course, they do not enter through the BBB, passage of substances across the BBB.

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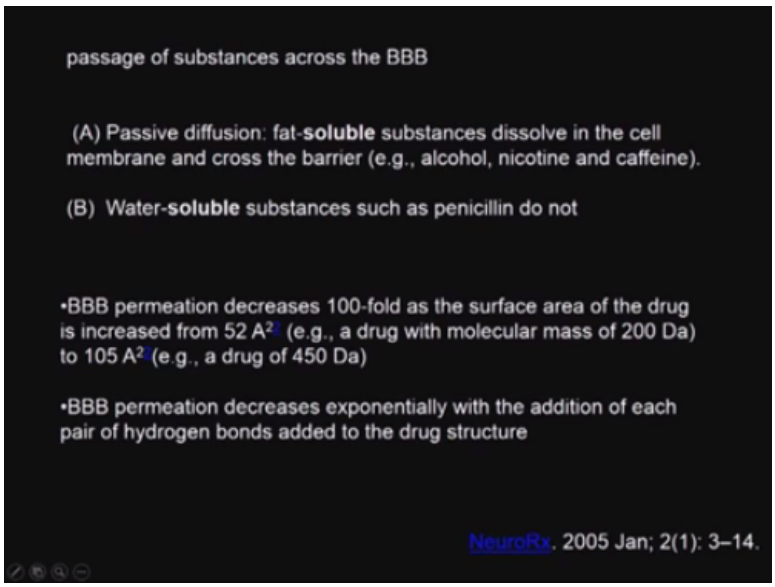

Like I said passive diffusion, fat soluble substances dissolved in the cell membrane and cross the blood brain barrier okay, example; alcohol, nicotine, caffeine if it gets dissolved in the fat, it can go. Water soluble substances such as penicillin, they do not okay. BBB permeation decreases 100 fold as the surface area of the drug is increased from 52 angstroms to 105 Angstrom.

So, if you increase double, then BBB permeation decreases by 100 fold. For example, a drug with the mass of 200 Dalton may have a surface area of 52 angstrom, whereas a drug with a 450 Dalton may have area of 105 angstrom okay, so it decreases. BBB permeation decreases exponentially with the addition of each pair of hydrogen bonds added to the drug. So, if you keep adding hydrogen bonds of course, you are increasing the hydrophilicity of the molecules.

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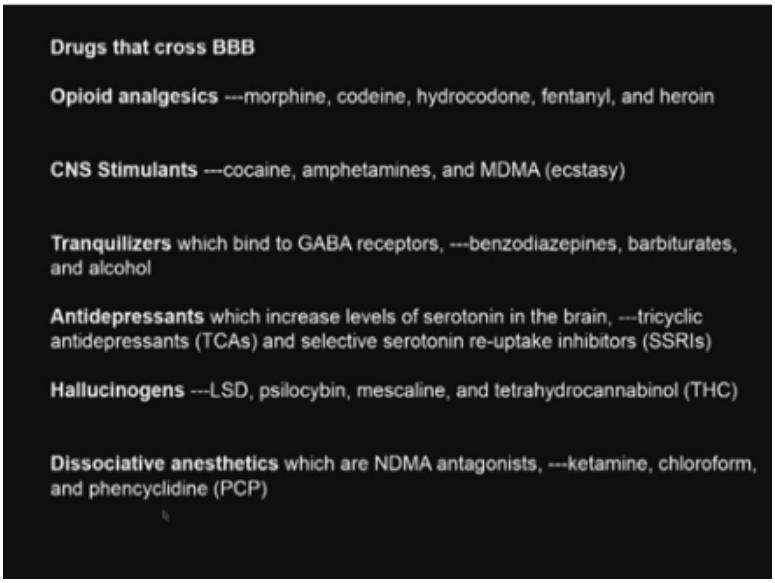

So, the permeation decreases exponentially, these are taken from this reference okay. So, what are the drugs that cross BBB? Like I mentioned, opioid analgesics; morphine, codeine, hydrocodone, fentanyl, heroin all these opioid analgesics they cross BBB. CNS stimulants; cocaine, just found in your coffee, amphetamines and MDMA ecstasy these are all drugs and then tranquilizers which bind to the GABA receptors like benzodiazepines, barbiturates, alcohol they all cross BBB.

Antidepressants, which increases levels of serotonin in the brain, tricyclic antidepressants and selective serotonin reuptake inhibitors so, they all cross BBB. Hallucinogens like LSD, psilocybin, mescaline, tetrahydrocannabinol, some of these cannabis type of drugs they cross BBB okay. Dissociative anaesthetics, which are NDMA antagonists; ketamine, chloroform and phencyclidine, they also cross BBB.

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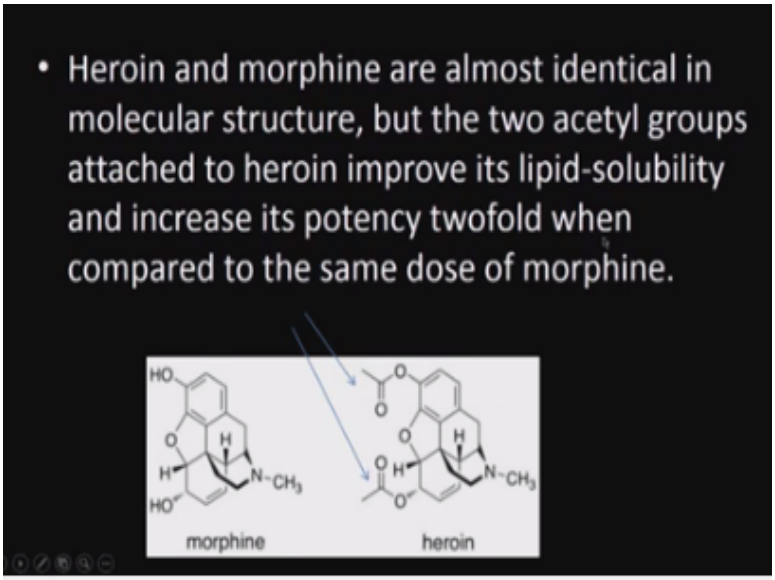

So, a lot of drugs cross this you know, opioid, stimulants, tranquilizers, antidepressants so you need also drugs to treat certain disorder, so you need some drugs which can cross the BBB okay, interesting look at this structure, this is called heroin, okay this is called morphine. If you look at Morphine and Heroin okay, Heroin has got extra acetyl group here as you can see with respect to, so there is an acetylation taking place, okay.

So, it becomes slightly more hydrophobic, so lipid solubility increases, so its potency is 2fold when compared to Morphine because it is become more lipid, so it is lipid soluble, so it can cross the BBB. So, Heroin has got 2-fold potency than Morphine, so slightly make the drug more lipid soluble, you increase the BBB penetration.

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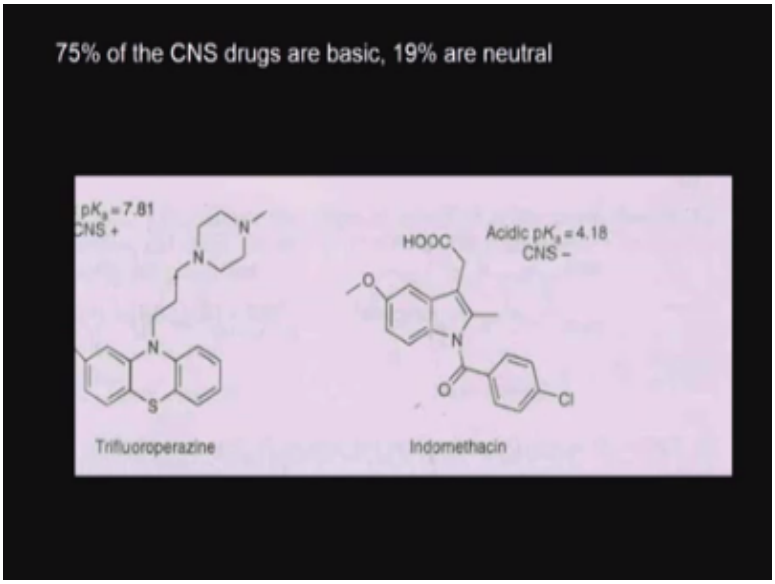

75% of the CNS drugs are basic okay, 90% are neutral, so basic drugs can penetrate BBB acidic ones will not okay, you have a basic drug when come acidic drugs, so these drugs can penetrate easily. So, if you are designing drugs for central neuro system, then look at more basic drugs okay, not acidic drugs because acidic drugs will not penetrate through the BBB, so that is one way of designing components for BBB penetration or preventing compounds from BBB penetrating okay.

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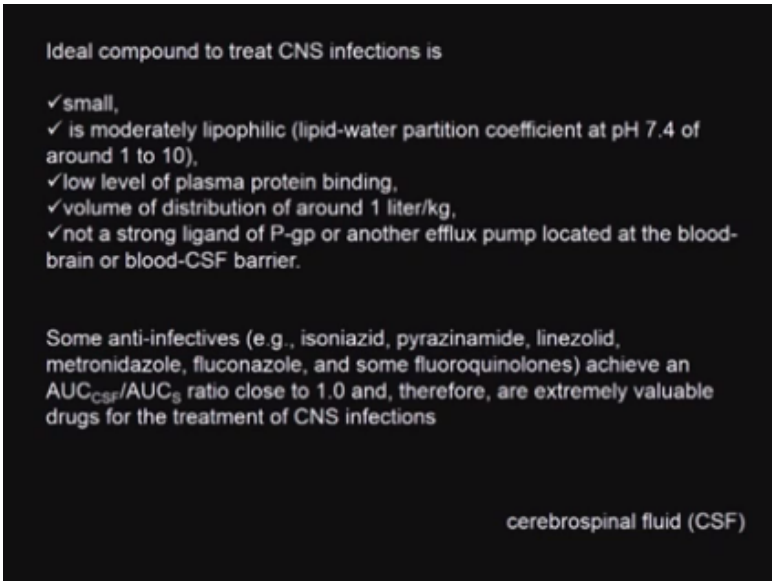

Ideal compounds to treat CNS infection that means if there are any infection in the brain region or if there are infection in the cerebrospinal region then we need to have a lot of anti-bacterial right, so molecule should be small is moderately lipophilic. So, lipid water partition coefficient at pH 7.4 of around 1 to 10, lower level of plasma protein binding because we do not want it to be a flexed out from BBB.

Volume of distribution of around 1 litre per kg not a strong ligand of Pgp, you do not want it to get a flexed out right, so it should not be a ligand of any of these efflux pump which are located at the blood brain or blood CSF that is cerebrospinal fluid barrier. For example, some anti infectives like tuberculosis; isoniazid, pyrazinamide, linezolid, metronidazole, fluconazole, and some fluoroquinolones achieve a good CSF to blood ratio okay 1.

Therefore, they are very valuable drugs for the treatment of CNS related infection, so that is the strategy one can think about small molecules moderately lipophilic, it should not; it should have low level of plasma protein binding, it should have; it should not be in ligand for Pgp efflux pump, the volume of distribution should be around 1 litre per kg body weight, okay.

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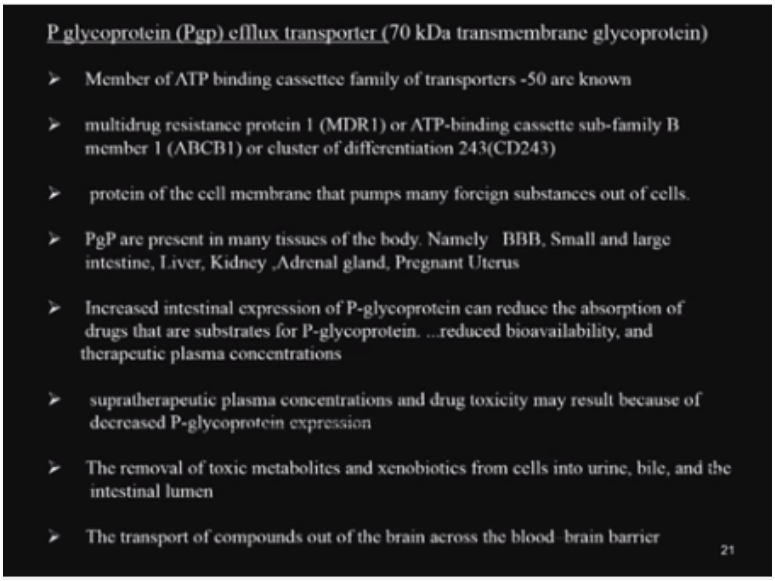

We have looked at now B glycoprotein sorry, we have looked at blood brain barrier as one of the important parameters to consider. Now, let us look at this P glycoprotein efflux transporter because it plays a very important role in effluxing drugs out of the system whether it is in the plasma region or whether it is in the blood brain region or any other part okay, we look; these are around 70 kilo Dalton transmembrane glycoproteins.

So, they throw compounds out of the system, so drugs may if they are get caught and they may get thrown out, so we need to design molecules which may have; which has low Pgp binding ability. What is this? These are the member of ATP binding cassette family of transporters, there are about 50 of them known and they are found in different parts of the body as for example, if you look at multi drug resistant diseases, you will find lot of these ATP's subfamily okay.

There are many different sub families and families are there, they pump out many foreign substances out of the cells okay, they are present in many tissues of the body namely BBB, small and large intestine, liver, kidney, adrenal gland, pregnant uterus, so the goal of it is to throw out any foreign substances which may be toxic to the system and so if the drug gets caught, it may also get thrown out.

Increased intestinal expression of P glycoprotein can reduce the absorption of drugs that are substrates for P glucoprotein okay, so it reduces bioavailability. So, if there are a lot of Pgp present in the intestine, so they get thrown out, so they get; does not get absorbed into the GI

and so bioavailability of the drug may be less, so supra therapeutic plasma concentrations and drug toxicity may result because of decreased P glycoprotein expression, okay.

If the glycoprotein expressions are low, then the amount of the drug may go up which may be leading to higher plasma concentration or even toxicity. So, people who may have large expression of Pgp may have different concentrations of the same drug in the plasma and those who have high expressions of Pgp may have lower concentrations of this particular drug in the plasma region okay.

The removal of toxic metabolites and xenobiotics from cells into urine bile and the intestinal lumen in fact, they are very important for us because they throw out all the toxic metabolites and xenobiotics from the cells okay, so they are doing a good job but the drugs also can get thrown out of the system because of the Pgp efflux action okay, the transport of compounds out of the brain across the blood brain barrier.

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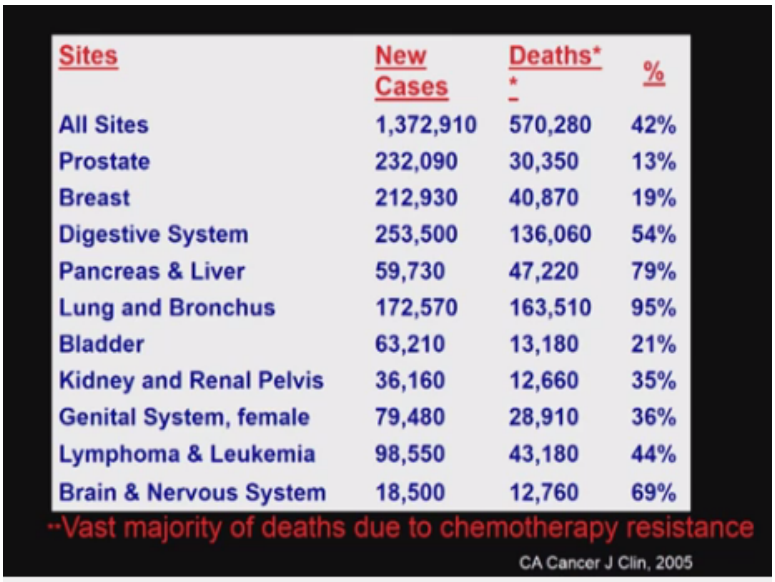

So, they also do an important job of throwing out like I mentioned in the previous section, the Pgp family are quite abundantly found in the BBB region okay. So, where are the sites? It is found in the prostate, all sites; 42%, 13% prostate, breast, digestive system is very high, pancreas and liver, lung bronchitis; bronchus, bladder and so on. So, they become chemo therapy resistance; the patients become chemotherapy resistance.

Because of okay, the high expressions of the Pgp and hence the drug resistance happens actually okay, so you can see many deaths happen because of the resistant to chemotherapy and here Pgp plays a very important role, okay.

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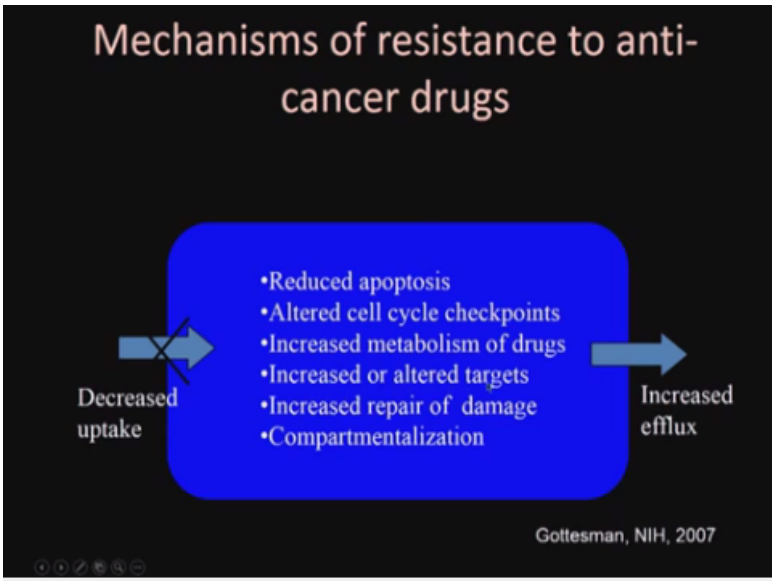

What are the mechanisms of resistance to anti-cancer drugs? For example, anti-cancer drugs there is an decreased in uptake in the cells, so there is a reduced apoptosis, altered cell cycle checkpoints, increased metabolism of drugs, increased or altered targets, increased repair of damaged, compartmentalization, so all these happen and also we have increased to efflux also, so decreased uptake, increased efflux, so all these are reasons why anti-cancer drugs become of no therapeutic value.

And Pgp plays a very important role especially in keeping the concentration of the drug to very low value inside the cells and hence the efficacy of the drug or the drug action becomes very minimal, so we need to understand the action of Pgp and we need to understand how or what type of molecules can become Pgp substrate and hence get thrown out and what type of molecules can escape this Pgp efflux action.

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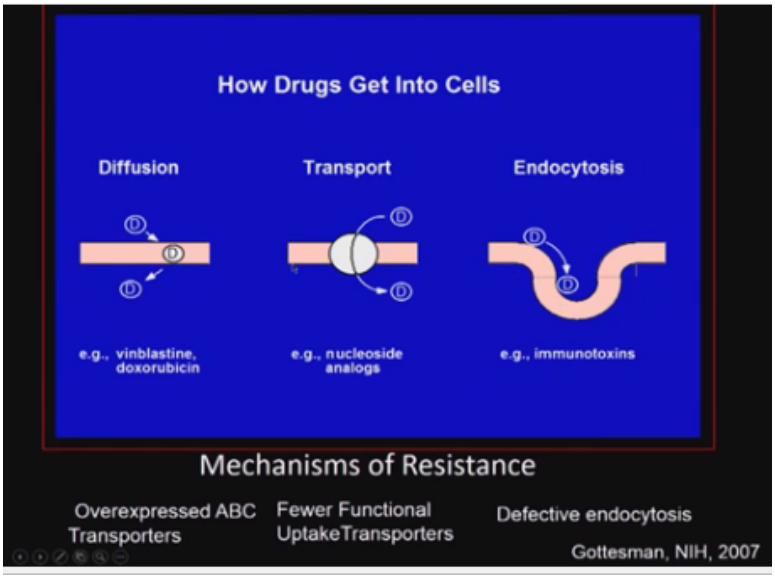

Thereby, if it can escape the Pgp efflux action, the concentration of the drug in the plasma region will be high and hence it may act effectively okay, so we will look more of this Pgp in the coming slides also. For example, if you look at how drugs get into cells; one is the diffusion okay like if you take anti-cancer drugs this is through vinblastine, doxorubicin diffusion. Transport okay, nucleoside analogues, so there are transporters which transport.

Endocytosis okay, so it is like gobbling up like all your immunotoxins, large molecules always go through this, large molecules cannot go to this diffusion and transport okay. So, if you have over expressed ABC transporters okay, you have fewer functional uptake transporters, defective endocytosis, so you could have problems leading to resistance okay because you are not that drugs do not penetrate and reach the inside of the cell.

This picture is taken from this reference given okay, so we will continue more on this topic of Pgp efflux pump transporters and how they play important role in the resistance acquired by cells and how we need to design drugs to escape this Pgp efflux transporters okay, thank you very much for your time.