**Enzyme Science and Technology**
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**Module - XII**
**Enzyme Applications (Part-II)**
**Lecture - 46**
**Enzyme in Drug Discovery**

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course, Enzyme Science and Technology.

And in this series of lectures, we so far what we have discussed? We have discussed about the basic information about the enzyme, we have discussed about the structure of the enzyme, we have also discussed about how you can be able to design and develop a new enzyme assay.

And we have also discussed how you can be able to study the enzyme substrate or the enzyme inhibitor interactions. And lastly, we have in the previous module, we were discussing about the application of the enzyme for the human welfare. So, in this context, we were discussing about how the enzymes are having the applications in the agriculture, PC culture, poultry, vaccine development, drug delivery, making the genetically modified organisms, medicines and transgenic animals.

So, if you recall, in the previous module, we have covered the two aspects of the enzyme applications. We have discussed about the enzyme application in the food industry. And then we have also discussed very briefly about the enzyme application in the medical field, how you can be able to use the enzyme for the diagnostic purpose. In today's lecture, we are going to discuss some more aspects related to the application of the enzyme in the to the for the human welfare.

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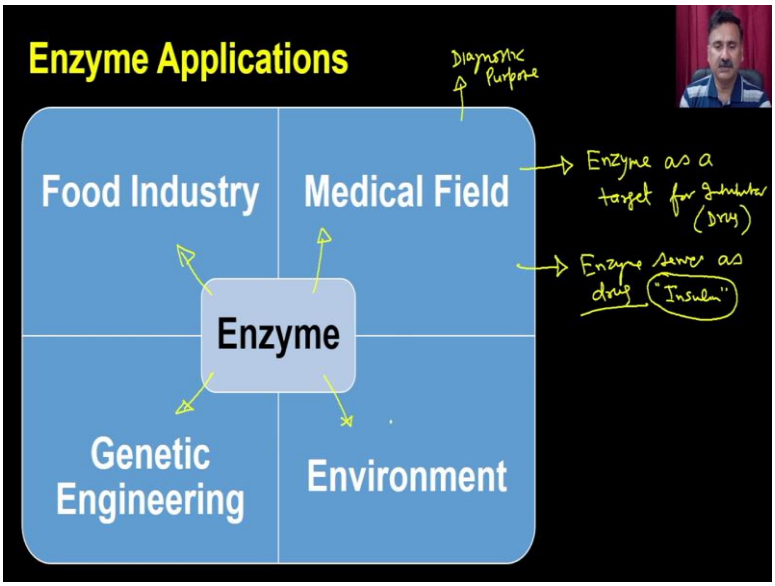


So, what you can see here is that the enzymes are actually having the diversified applications, they are actually having the applications in every other fields of biotechnology where you are actually supposed to convert a product into substrate into product or something like that.

But what we have chosen the only the four topics, application of enzyme in the food industry or enzyme in the medical industry or environment and as well as the enzyme in the genetic engineering. Within the medical field, we have discussed about how you can be able to use the different types of enzyme for the diagnostic purposes.

So, we have discussed about how you can be able to use the level of different types of enzyme in the serum such as alkaline phosphatase or SGOT, SGPT and all those kinds of things. And how you can be able to use that information to predict the damages into the particular type of tissue or organ and based on that, you can actually be able to you know diagnose the disease and other kind of things.

The other aspect of the enzyme is that where the enzymes are actually going to serve as a target. So, target for you know, for inhibitor development. So, these inhibitors would be nothing but the drug molecules, right. So, these drugs will actually inhibit the enzyme. These enzymes are catalyzing a particular reaction, which are actually disturbing the physiology of the organisms and so on. So, you can actually be able to inhibit this enzyme with the help of a drug.

Remember that in the past, we have discussed about how you can be able to use the different approaches to design the inhibitors, whether it is the traditional approach or the computer aided drug design approach, whatever the approach could be, you can be able to use these approaches to design the inhibitor, drug molecule. And that drug molecule, you can be able to use to inhibit this particular enzyme.

The other aspect is that the, where the enzyme itself serve as drug, ok. So, in some of the cases where you have the genetically a deficiency, genetic deficiency of particular enzyme, ok. So, the when the organism grow, the requirement of that particular enzyme is very high.

And in that cases, you may not be able to have the sufficient quantity of that enzyme. And that is how you will not be able to convert the substrate into the product. And as a result, there will be an accumulation of the substrate at one end and there will be a deficiency of the product. And that product is actually required for running the metabolisms.

So, in that cases, you are actually going to take this enzyme as a supplement. Either you are going to take this enzyme through as a tablet or you are going to take this enzyme as the injections and so on. Classical example is the insulin, right. So, insulin is hormone, right.

So, it is just to work like that way, ok. So, same way you can actually have the enzyme also. And then we have also going to discuss, in today's lecture, we are also going to discuss about the role of the enzyme in the environment and genetic engineering.

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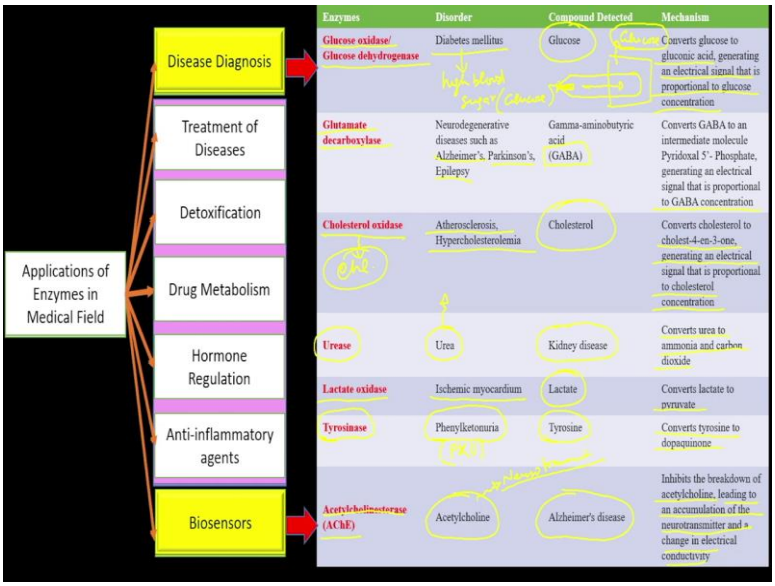


Now, let us talk about the application of the enzyme in the medical field. So, as I said, you know, we enzymes can be used for disease diagnostics or biosensors. They can be able to use for the treatment of the disease, detoxifications, drug metabolisms, hormone regulations and anti inflammatory agents. So, as far as the disease diagnosis is concerned, you can actually be able to use the different types of enzymes for the detection purposes.

For example, we can use the glucose oxidase or glucose dehydrogenase. These are the two different enzyme what you can actually use. And that can be used to detect the diabetes mellitus. You know that the diabetes mellitus is a disease where you are actually going to have the high blood sugar. So, sugar means glucose, ok. So, in this particular case, you are going to use the glucose oxidase or dehydrogenase depending upon the different types of products. And you are actually going to detect the compound.

And the mechanism through which it actually detects the glucose is that the glucose is actually going to be get converted into the gluconic acid, right. And it is actually going to generate a electrical signal and that is proportional to the glucose concentrations. So, what you are going to have is you are going to have.

I am sure you might have seen some of your older people in your family that how they are actually using the glucose strips detecting the glucose. So, that glucose strip what you

see in your home right, that actually has something like this, right. And you have a you know some place here to place the blood, right.

And within this and then you insert this glucose strip into a glucometer, right. And what happens is that when you strip, when you put the blood on or drop of blood onto this, it actually goes inside through a capillary action. And then the glucose is actually whatever the amount of glucose is present in that particular amount of blood is actually going to be get converted into gluconic acid.

And in this process, it is actually going to generate the electrical pulse means, it actually going to generate a the current actually. And that current is actually going to measured by the glucometer with the help of a meter, right. And that signal is actually going to be processed by a calibration curve. And as a result, it is actually going to tell you what is the amount of glucose concentration, right.

Because they have already calibrated the machine with the help of standard glucose solutions and so on. So, that is how it is actually going to give you the accurate values. Then the second example is the glucose glutamate decarboxylase it is this is also another enzyme which is actually going to use for detection of the neuro degenerative diseases such as Alzheimer disease, Parkinson disease or the epilepsy.

And the with the help of the glutamate decarboxylase, you are actually going to detect the level of gamma aminobutyric acid. And the mechanism is that the GABA is going to be converted into a pyridoxal 5 phosphate generating a elliptical signal and that is proportional to the GABA concentrations. So, mechanism is remained the same as what we have discussed for the glucose except that the enzyme is different and the detection, the analytes is also different.

Then we have the cholesterol oxidase. So, cholesterol oxidase is a enzyme which is actually going to be used for detection of the two diseases, the atherosclerosis or the hypercholesterolemia. Basically, the cholesterol oxidase is going to allow you to detect the cholesterol, right.

So, it is actually going to allow you to detect the cholesterol, what is present into the particular biological fluid. And how it detects? It converts the cholesterol into the

cholesterol-4-en-one and it generates the electrical signal and that is proportional to the cholesterol concentrations.

Then we have the Urease. And Urease is a very, very important enzyme. It is one of the very old enzyme, what is being isolated by the scientists. And urease is actually going to allow the detection of the compound which is called as urea. And when you have a high quantity of urea into the blood it actually going to give you the detection of the kidney diseases, ok.

And how the urease is actually work as the diagnostic marker or as a molecule to detect the urea is that it converts the urea into ammonia and carbon dioxide and you can actually be able to you know, the measured the level of ammonia and carbon dioxide and that is how you can be able to measure the level of urea. Then we have the lactate oxidase. So, lactate oxidase is actually going to give you the detection of the ischemic myocardium and it actually detects the lactic acid, right.

And the mechanism is working is that it converts the lactate into the pyruvate and in this process, it is actually going to detect the lactate amount of lactate present in the biological fluid. Then we have the tyrosinase. Tyrosinase is a enzyme which actually going to help you to detect the genetic disease called phenylketonuria or PKU, right. And it detects the tyrosine, level of tyrosine because when there will be a disturbance of the phenylalanine metabolism it is actually going to accumulate the large quantity of tyrosine.

So, it converts the tyrosine into the dopaquinone and in this process it is actually going to help you to detect the level of tyrosine present in the biological fluid. And then we have the acetylcholinesterase, acetylcholinesterase is an enzyme which actually helps to detect the acetylcholine level. And acetylcholine if you recall it is a neurotransmitter, right.

So, it is a neurotransmitter and if the there will be a depletion or reduction in the level of neurotransmitter, it is actually going to disturb the neural the conduction of the neural signal. And as a result, the person is actually going to have the neural degenerative diseases or it is actually going to indicate it is a clear indication that the person is having the brain related actions.

So, that is actually going to help you detect the Alzheimer disease. And how it works is that acetylcholinesterase inhibits the breakdown of the acetylcholine and leading to the accumulation of a neurotransmitter and a change in electrical conductivity. And as a result, you can actually be able to measure the level of acetylcholine.

Now, what we are going to do is we are going to also understand that how the enzymes are actually helping to design the different types of drugs, right. We have taken couple of diseases against which how the people have actually developed the drug molecules.

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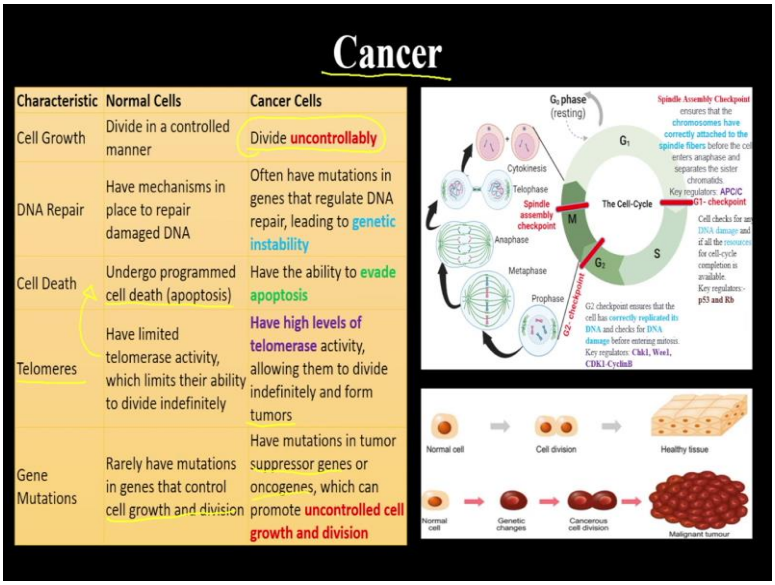


So, the first disease what I have taken is actually the cancer, ok. So, before getting into the detail of the cancer and you know how the people are developing the drugs and all that, what you have to understand is that, the cancer is a disease where you are actually going to have the uncontrolled division of the cell, right. So, this is I have just given an example of what is the normal cell look like and what are the features of the normal cells and what is the feature of the cancer cells.

Then normal cell have the limited capacity to repair the damaged DNA, right. And as a result, it is actually going to undergo the process of apoptosis whereas; the cancer cells have the inhomorous capacity to do the DNA repair. And as a result, if they can be able to overcome all the damages what is happening into the genomic content and that is how they are actually going to be survived after the damage happened.

Then the cell death. So, normal cells actually undergoes a normal cell death which is called as the apoptosis whereas, the cancer cells could be able to escape this process and that is how they are immortal and they will be able to grow for the several generations. In the level of telomeres.

So, the normal cells have the have the limited telomeres activity and that is how they are actually going to have the loss of the genomic content after every cell cycle division. And as a result, only they are actually going to undergo to the apoptosis. Whereas in the case of the cancer cells they have a very high level of telomerase activity which allow them to repair the telomeres and as a result they will be able to go divide indefinitely and form the tumors or the solid mass.

Then the gene mutations so, the normal cells are actually having the mutations rarely have the mutation in genes and that controls the cell growth and the division. Whereas, in the tumor cells they have the mutations in tumor suppressor genes or the oncogenes and which can promote the uncontrolled cell death and growth.

So, these are the some of the things what we have shown that how the cell cycle is happening and how the different stages of the cell cycle is happening and how the different molecules or the enzymes are participating in these pathways and how the normal cell is getting converted into the cancer cell is that the normal cell is going through the cell division and it is forming the healthy tissue.

Whereas, the normal cell when it is going through the genetic changes which means the when it is going through the mutations and other things it is actually acquiring the transformogenic phenotype and as a result it is actually going to form the malignant tumor.

So, malignant tumor is also going to be the same cell, but it is not going to be functional cells which means for example, if we start with liver cells for example, if we are talking about the liver cancer these are the liver cells these are also liver cells they will grow and give you a liver, right. They so, these are the hepatocytes actually they will go and go and divide and then it will give you a liver, right whereas, in the case of the cancer cells they will actually be hepatocytes.

They will grow, they will not going to give you the liver, they will actually going to give you the tumor. And these tumor cells are actually going to take up the space what is being taken up by the healthy cells. And as a result, the amount of the amount of the a healthy cells is going to be keep reducing over the course of time. And as a result, at the end, it is actually going to start giving the phenotypic changes into the human or into the organisms.

So, the cancer cell the major issue with the cancer cell is that they are having these extraordinary growth profiles; they are actually going to sustain the different types of damages. And at the end the these cells are not going to perform the normal functions, they are actually going to only take up the nutrition and grow, right.

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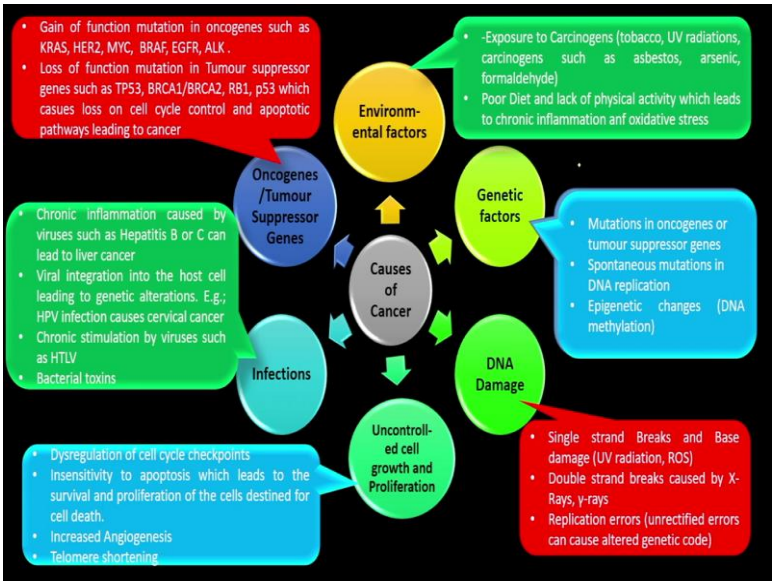


Now, these are the some of the causes of the cancer. So, you can have the cancers because of the environmental factors, you can have genetic factors, DNA damage, on uncontrolled cell growth and proliferation and in the infections. We are not going to discuss each of these aspects because the course is all about the enzymes and the how you can be able to use the enzyme for developing the drug.

So, but I have you know I have given you in the slides. So, that you can if you are interested you can be able to study some of these aspects and you can be able to follow the content. So, our cancer get developed into a human because of the accumulation or because of the even the combination of these factors not like only the environmental

factor is going to cause the cancer or genetic factors are going to cause the cancer, it could be a combination which actually will give the equilibrium toward the development of cancer cells.

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| Drug Class | Examples of Drugs | Mechanism of Action | Targeted Enzyme | Role of Enzyme |
|---|---|---|---|---|
| Alkylating agents | Cyclophosphamide, cisplatin, carmustine | Cross-link DNA to prevent replication and induce apoptosis | DNA polymerase | Adds nucleotides to DNA strands during replication |
| Antimetabolites | 5-fluorouracil, methotrexate, gemcitabine | Disrupt DNA synthesis and repair by mimicking naturally occurring nucleotides | Thymidylate synthase, dihydrofolate reductase, DNA polymerase | Synthesizes nucleotides needed for DNA replication and repair |
| Topoisomerase inhibitors | Irinotecan, etoposide, doxorubicin | Inhibit the activity of topoisomerases, enzymes involved in DNA replication and repair | Topoisomerase I, Topoisomerase II | Relieves tension in DNA strands during replication and repair |
| Mitotic inhibitors | Paclitaxel, vincristine, docetaxel | Disrupt microtubules, preventing proper chromosome segregation during cell division | Tubulin | Forms microtubules that are required for proper cell division |
| DNMT inhibitors | Decitabine, azacitidine, guadecitabine | Inhibit DNA methyltransferases (DNMTs), enzymes that add methyl groups to DNA | DNMT1, DNMT3A, DNMT3B | Regulate gene expression by adding methyl groups to DNA |

So, these are the some of the drug molecules what are being developed. So, you can have the alkylating agents, you can have the anti-metabolites, you can have topoisomerase inhibitors, you can have mitotic inhibitors and then you also have DNMT inhibitors.

So, you can have the drugs like alkylating agents, you can have the cyclophosphamide, cisplatin and carmustine. So, it actually cross link the DNA to prevent the replication and induce and the target enzyme is the DNA polymerase right, DNA polymerase which is enzyme which is involved in these DNA replication, right.

So, it is actually going to stop the DNA replication. And once the there would be a stoppage of DNA replications it is actually going to not allow the cell to grow beyond the S phase. So, it will actually the cells will stuck into the S phase and as a result they will not be able to complete the cell cycle and ultimately they will actually going to die.

Then the anti-metabolites you can have an example of 5-fluorouracil, methotrexate and gemcitabine. It disrupt the DNA synthesis and repair by mimicking the naturally occurring the nucleotides the enzymes the target enzyme is thymidylate synthase, dihydrofolate reductase and DNA polymerase.

Apart from that you remember that when we are talking about this, we have also said that SHMT serine hydroxy methyltransferase. And why I always mention the SHMT because the SHMT is an enzyme for which I have solved the structures and I have actually did my thesis on that. So, that is why I always mention that although SHMT is not that main target for the 5-fluorouracil methotrexate, it synthesizes the nucleotides needed for the DNA replications and repair, right. So, it actually blocks the DNA synthesis, right.

Then we have topoisomerase inhibitors. So, we can have the etoposides, you can have the doxorubicin, you can have irinotecan and it is inhibit the activity of topoisomerase which is involved in the DNA replication and repair. So, the enzyme is topoisomerase 1 or topoisomerase 2. And release the tension in the DNA strands during the application and repair.

Then we have the mitotic inhibitors. So, paclitaxel, vincristine and docetaxel and it disrupt the microtubules preventing the proper chromosomal segregation. And the target molecule is the tubulin proteins and it forms a microtubule that are required for the proper cell divisions.

Then we have DNMT inhibitors. So, the DNMT inhibitors are going to inhibit the DNA methyltransferase enzyme that adds a methyl group to the DNA and the enzymes are DNAMT1, DNAMT3A, DNAMT33B and it regulates the gene expression by adding the methyl group to the DNA.

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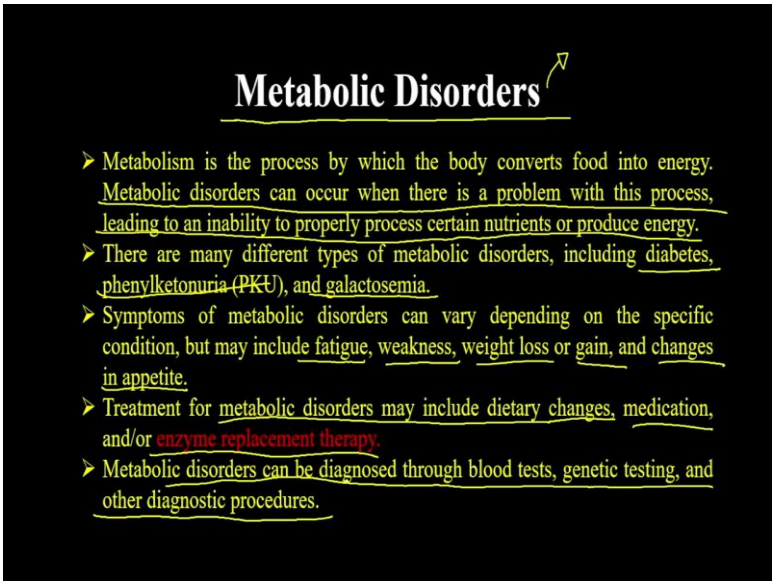

Then we have taken another example of the drug which is of the diseases where you have the metabolic disorders. Metabolic disorder means the diseases which are not been which are where you are actually going to have the disturbance of the metabolites. So, metabolism is a process by which the body converts the food into energy, right.

So, metabolic disorder can occur when there is a problem with the process leading to an ability to properly process certain nutrients or the produce energy. There are many different types of metabolic disorders such as diabetes, phenylketonuria and the galactosemia.

Symptoms of the metabolic disorder can vary depending upon the specific condition, but may include the fatigue, weakness, weight loss and gain and changes in the appetite. Treatment for metabolic disorder may include the dietary changes, medications and the enzyme replacement therapy. Metabolic disorder can be diagnosed through blood tests, genetic testing and the other kind of diagnostic features.

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| Disorder | Defective Enzyme | Role of Enzyme | Therapy |
|---|---|---|---|
| Phenylketonuria (PKU) | Phenylalanine hydroxylase | Converts phenylalanine to tyrosine | Low phenylalanine diet, BH4 supplementation, Kuvan (drug), gene therapy (in development) |
| Galactosemia | Galactose-1-phosphate uridyltransferase | Converts galactose-1-phosphate to glucose-1-phosphate | Galactose-free diet, monitor blood glucose, calcium and vitamin D supplementation |
| Maple syrup urine disease (MSUD) | Branched-chain alpha-ketoacid dehydrogenase complex | Converts branched-chain amino acids to their corresponding alpha-ketoacids | Low-protein diet, thiamine supplementation, liver transplant |
| Pompe disease | Acid alpha-glucosidase | Breaks down glycogen to glucose | Enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase (rhGAA), gene therapy, supportive care |
| Fabry disease | Alpha-galactosidase A | Breaks down glycosphingolipids, including globotriaosylceramide (Gb3) | Enzyme replacement therapy (ERT) with agalsidase alfa or beta, chaperone therapy, gene therapy (in development) |
| Gaucher disease | Glucocerebrosidase | Breaks down glucocerebroside | Enzyme replacement therapy (ERT) with imiglucerase, velaglucerase alfa or taliglucerase alfa, substrate reduction therapy, bone marrow transplant |
| Tay-Sachs disease | Hexosaminidase A | Breaks down GM2 ganglioside | Supportive care, symptom management, gene therapy (in development) |

So, we have taken a few examples. So, so we can have the first example there is a phenylketonuria. Phenylketonuria the is a genetic disease where you can have the problem of phenylalanine hydroxylase, the enzyme which is responsible for you know metabolizing the phenylalanine and the role of this enzyme is that it converts the phenylalanine to tyrosine, right and as a result that there will be a tyrosine. Tyrosine which is actually going to be utilized.

So, low phenylalanine diet what is the therapy? Therapy is that you take you do not take the phenylalanine, because this enzyme is not present, right. So, it will not be able to convert the phenylalanine to tyrosine. And as a result, there will be an accumulation of the phenylalanine. So, what you, so therapy is that you take the low phenylalanine diet or you can take the BH4 supplementation or kuvan gene therapy in development, ok.

So, you can actually there are reports where you can actually be able to have the gene therapy for phenylalanine hydroxylase and that (Refer Time: 24:07) also giving the relief to the patients. Then we have the galactosemia. So, galactosemia, the defective enzyme is the galactose-1-phosphate uridyltransferase. And the role of the enzyme is that it converts the galactose-1-phosphate to glucose-1-phosphate.

The therapy is that you take the galactose free diet, monitor the blood glucose levels, calcium and vitamin D supplementations. Then we have the maple syrup urine disease. So, there you have the this particular defective enzyme that called branched chain alpha-keto acid dehydrogenase complex. And that converts the branched chain amino acid to their corresponding alpha-keto acids, ok. And the therapy is that you take the low protein diets and thiamine supplements and the liver transplants.

Then we have the pompe disease. So, the defective enzyme is the acid alpha glucosidase. It break down the glycogen to the glucose. And the therapy is that you take the enzyme replacement therapy with recombinant human alpha-glucosidase and gene therapy and the supportive cares Fabry disease.

So, the defective enzyme is the alpha glucosidase, alpha galactosidase it break down the glycosphingolipids including the globotriaosylceramides or Gb3. And the therapy is that the enzyme replacement therapy with the agalsidase alpha or the beta and the chaperone therapy and then gene therapy.

Then we have the gaucher disease. So, gaucher disease is the enzyme which is called as glucocerebrosidase. And it converts the, it actually breaks down the glucocerebrosidase, right. And the therapy is that you are have the enzyme replacement therapy with the imiglucerase alfa or this particular enzyme.

And then you have the substrate reduction therapy or the bone marrow transplant. Then we have Tay-Sachs disease where you have the Hexosaminidase A, it break downs the

GM2 ganglioside remember then we were talking about the lipids. We have discussed some of these lipids supportive cares, symptoms management and gene therapy.

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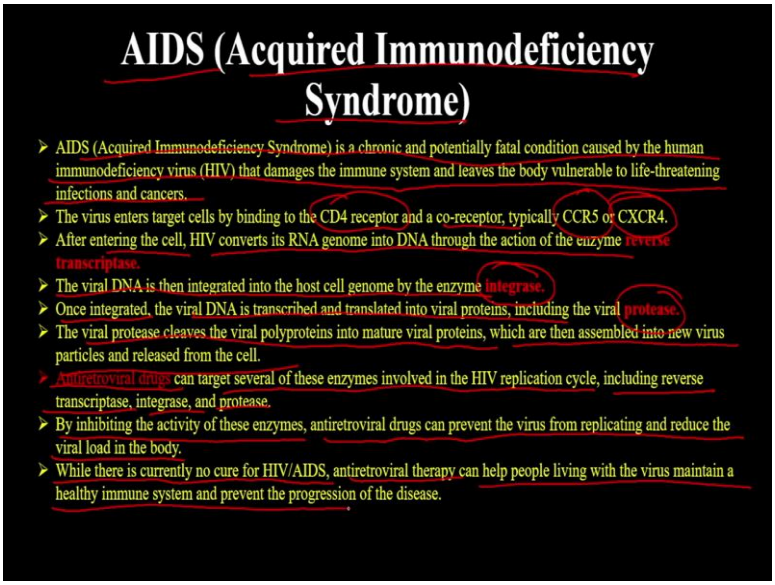


Then we also taken the infectious disease. So, one of the infectious disease what I have taken is the aids or the acquired immunodeficiency syndromes. And the aids is a chronic and potentially fatal condition caused by the human immunodeficiency HIV that damages the immune system and leave the body vulnerable to the life-threatening infections and diseases.

The virus enters the target cell by binding to the CD4 receptors and the co-receptors typically CCR5 or CXCR4. After the entering the cell, HIV converts its RNA genome into DNA to the action of enzyme which is called as reverse transcriptase. The viral genome is then integrated into the host genome by enzyme which is called as integrase. Once integrated, the viral the DNA is transcribed and translated into the viral protein including the viral protease.

The viral protease cleaves the viral polypepta poly proteins into the mature viral genome which then assemble into the new virus particle and released from the cell. Anti-viral drugs can target the several of these enzymes involved in the HIV replications including the reverse transcriptase, integrase and protease. By inhibiting the activity of these enzyme, antiretroviral drug can prevent the virus from replicating and reduce the virus load into the body.

While there is a currently no cure for HIV and AIDS, antiviral therapy can help the people living with the virus maintain the healthy immune system and prevent the progression of the disease.

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| Target Enzyme | Role of Enzyme | Drug Class | Examples of Drugs | Mechanism of Action |
|---|---|---|---|---|
| Reverse Transcriptase | Converts viral RNA to DNA during the viral replication cycle | Nucleoside Reverse Transcriptase Inhibitors (NRTIs) | Zidovudine, Lamivudine, Emtricitabine | Inhibit viral replication by blocking the conversion of RNA to DNA |
| Reverse Transcriptase | Converts viral RNA to DNA during the viral replication cycle | Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) | Efavirenz, Nevirapine, Delavirdine | Inhibit viral replication by binding to and altering the structure of the enzyme |
| Protease | Cleaves viral polyproteins into mature viral proteins during the viral replication cycle | Protease Inhibitors (PIs) | Saquinavir, Ritonavir, Atazanavir | Inhibit viral replication by blocking the cleavage of viral polyproteins into mature viral proteins |
| Integrase | Integrates viral DNA into the host cell genome during the viral replication cycle | Integrase Inhibitors | Raltegravir, Elvitegravir, Dolutegravir | Inhibit viral replication by blocking the integration of viral DNA into the host cell genome |
| gp41 | Facilitates fusion of viral and host cell membranes during viral entry | Fusion Inhibitors | Enfuvirtide, Maraviroc | Inhibit viral replication by blocking the fusion of viral and host cell membranes |

So, these are the some of the enzymes, what you can actually be able to target, if you want to target the if you want to treat the HIV or the AIDS. If you can have the reverse transcriptase, the reverse transcriptase, the you can actually be able to use the nucleoside reverse transcriptase inhibitors. The examples are zidovudine, lamivudine and emtricitabine and it inhibits the replication by blocking the conversion of RNA to DNA.

Then we can also use the reverse transcriptase and you can actually be able to use these inhibitors and they inhibit the replication by binding to an alterating the structure of the enzyme. Then you can also target the viral protease. So, that viral protease, you when you put the protease inhibitors such as the atazanavir and ritonavir and inhibits the DNA replication viral replication by blocking the cleavage of the viral polypeptide into the mature viral proteins.

Then you can also inhibit the integrase. So, integrase is going to be inhibit by the integrase inhibitors. So, these are the examples right, of the inhibitors and it inhibits the viral application by blocking the integration of the viral DNA into the host cell genome.

Then you also have the gp41. So, gp41 is a you can use the fusion inhibitors and these are the two inhibitors, what you can actually be able to use. So, these are the two drugs, what are circulation. And it inhibits the viral replications by blocking the fusion of the viral and the host cell membranes.

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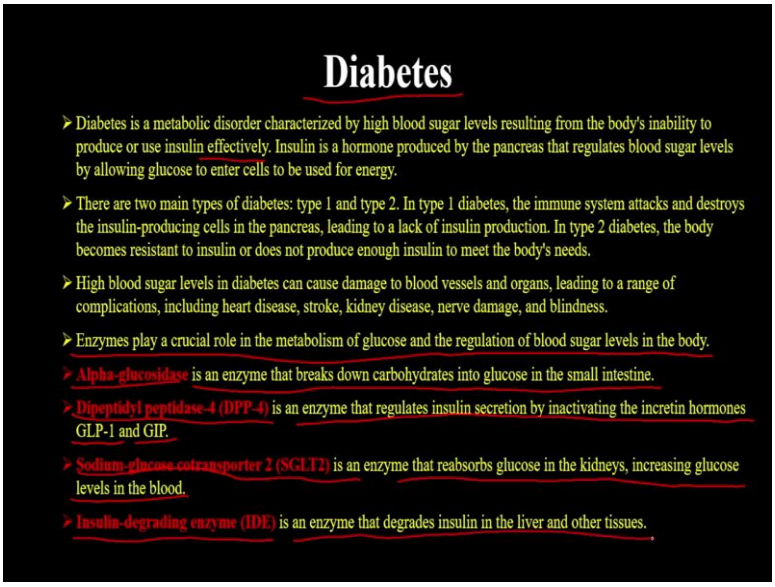


Then we have the another disease which is called as diabetes. So, diabetes is a metabolic disorders characterized by the high blood glucose level resulting from the body's inability to produce or use insulin effectively, right. And we have the several cheesing, several things what you can actually be able to use.

So, enzymes play a crucial role in the metabolism of glucose and regulation of the blood glucose level in the body. So, alpha glucosidase is an enzyme that breakdowns the carbohydrate into glucose in the small intestine. Then we have the dipeptidyl peptidase is an enzyme that regulates the insulin secretion by inactivating the secretin hormones like GlP-1 and GIP.

Then we have the sodium glucose cotransporters is an enzyme that reabsorbs the glucose into the kidney increasing the glucose level in the blood. And then we also have the insulin degrading enzyme and it is an enzyme that degrades the insulin into the liver and other tissues.

So, these are the four enzymes which are actually playing a very crucial role in regulating the blood glucose level. So, if you want to develop the drug you can actually be able to modulate their activities. And as a result, there will be able to you can be able to you know control the blood glucose level.

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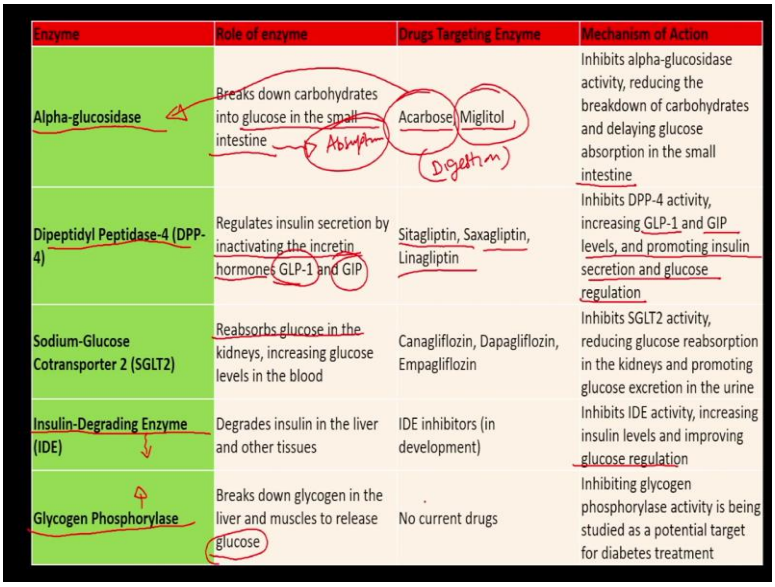

| Enzyme | Role of enzyme | Drugs Targeting Enzyme | Mechanism of Action |
|---|---|---|---|
| Alpha-glucosidase | Breaks down carbohydrates into glucose in the small intestine | Acarbose, Miglitol | Inhibits alpha-glucosidase activity, reducing the breakdown of carbohydrates and delaying glucose absorption in the small intestine |
| Dipeptidyl Peptidase-4 (DPP-4) | Regulates insulin secretion by inactivating the incretin hormones GLP-1 and GIP | Sitagliptin, Saxagliptin, Linagliptin | Inhibits DPP-4 activity, increasing GLP-1 and GIP levels, and promoting insulin secretion and glucose regulation |
| Sodium-Glucose Cotransporter 2 (SGLT2) | Reabsorbs glucose in the kidneys, increasing glucose levels in the blood | Canagliflozin, Dapagliflozin, Empagliflozin | Inhibits SGLT2 activity, reducing glucose reabsorption in the kidneys and promoting glucose excretion in the urine |
| Insulin-Degrading Enzyme (IDE) | Degrades insulin in the liver and other tissues | IDE inhibitors (in development) | Inhibits IDE activity, increasing insulin levels and improving glucose regulation |
| Glycogen Phosphorylase | Breaks down glycogen in the liver and muscles to release glucose | No current drugs | Inhibiting glycogen phosphorylase activity is being studied as a potential target for diabetes treatment |

So, if you want to target the alpha glucosidase you can actually you know the role of enzyme is that it is actually going to break down the carbohydrate into glucose in the small intestine. And as a result, it is actually going to allow the absorption of the glucose molecule.

So, if you use the molecules like acarbose or miglitol these are the two inhibitors actually of the alpha glucosidase and it inhibits the alpha glucosidase activity reducing the breakdown of the carbohydrate and delaying the glucose absorption into the its small intestine. This means you are indirectly by to putting these two molecules or you are indirectly disturbing the carbohydrate digestions, ok.

So, you are actually targeting the digestion. And as a result, the there will be no digestion of the carbohydrate into the glucose monomers. And the once there the carbohydrate is not going to be a degrade into the glucose monomer, they will not going to be absorbed into the small intestine.

Then we have the dipeptidyl dipeptidase. So, it regulates the insulin secretion and you are supposed to increase the you know so it is regulates the insulin secretion by inactivating the hormone secreting hormones like the GLP-1 and GIP. So, what you can do is you can actually be put the these molecules, right. And they will be able to inhibit the DPP-4 activity increasing the GLP-1 and GIP levels and promoting the insulin secretion and the glucose metabolism.

Then we have the sodium glucose co-transporters. So, it actually allows the reabsorption of the glucose from the urine right, into the kidney. So, you do not want the reabsorption, right. So, what you can do is, you can just put these inhibitors and they will inhibit the activity of this particular transporter. And as a result, there will be a loss of blood sugar or loss of glucose into the urine and it actually eventually going to help in reducing the level of sugar.

Then we have insulin degrading enzyme. So, insulin degrading enzyme as the name suggest its actually going to degrade the level of insulin into the blood. And it is actually required because it actually controls the level of insulin into the blood. But if the insulin degrading enzyme activity will go up, it is actually going to not have the it will not allow the sufficient glucose into a sufficient insulin into the blood glucose.

So, what you can do is you can put the IDE inhibitors and they will inhibit the IDE activity increasing the insulin level and promoting the glucose regulations. Then we have the glycogen phosphorylase. So, glycogen phosphorylase breakdowns the glycogen in the liver and muscle to release the glucose, right.

So, it is actually going to the reverse, right. So, if you inhibit this it is actually going to reverse the things right, it is actually going to allow more glucose into the form of glycogen and the form of rather than in the form of glucose and (Refer Time: 34:15) that it is actually going to reduce the level of glucose into the blood glucose.

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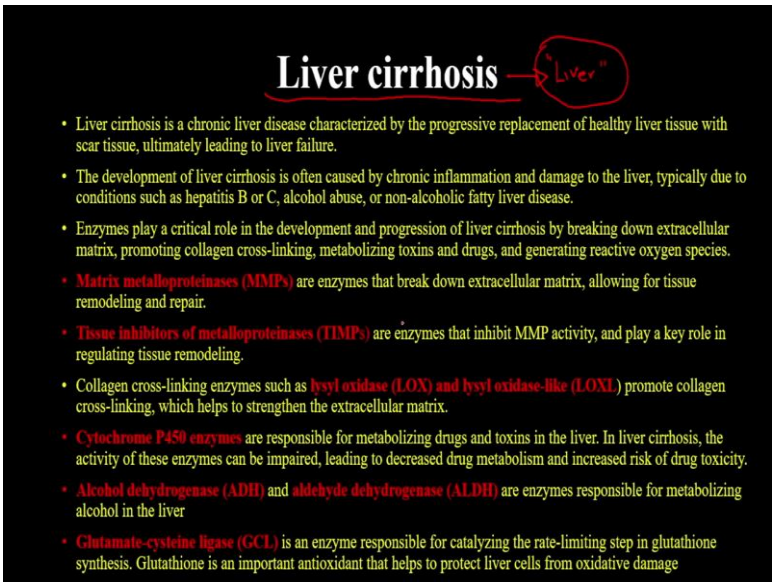


Then we have the liver cirrhosis. So, liver cirrhosis is a disease of the liver where the liver is actually going to have the. So, liver cirrhosis could be non infectious, liver cirrhosis could be infectious. So, the there are so many mechanisms what we are actually going to happen and how the liver cirrhosis is going to affect the human metabolism and all that, but we are not going to discuss that. This is all what we have said and what we are going to give you in terms of you are interested to study about the liver cirrhosis.

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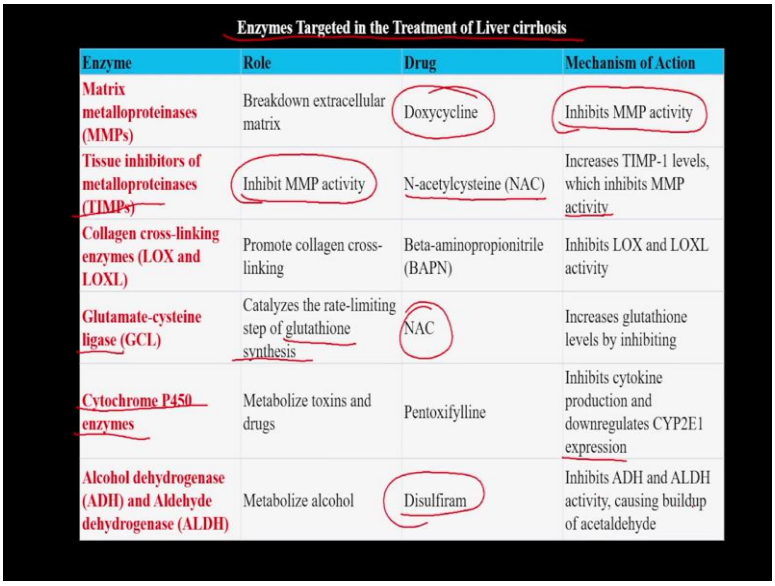

What we are talking about is the you know the enzymes which are target which are being used to the in as a target in the treatment of the liver diseases. So, you can have the different types of MMPs, you can actually which are going to have the role of break down of the cellular matrix and you can actually be able to use the drug which is called as doxycycline and it inhibits the MMP activity. So, once you have the loss of the MMP activity it is actually going to help enter the liver cirrhosis.

Then we have the TIMPs. So, TIMPs are actually the enzyme, which are inhibiting the MMP activity. So, you can actually be able to use the N-acetylcysteine NAC and it increases the TIMP level which inhibits the MMP activity. Then you have the collagen cross linking enzyme, LOX and LOXL and that promotes the collagen cross linking.

You can actually be able to use the beta amino propionitrile and it inhibits the LOX and LOXL inhibitors. Then we have the glutamate cysteine ligase and you can actually be able to catalyze the rate limiting step of glutathione synthesis and you can actually be able to put the N-acetylcysteine, NAC and that increases the glutathione level by inhibiting the this enzyme.

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**Enzymes Targeted in the Treatment of Neurodegenerative Diseases**

| Enzyme | Role in neurological disorders | Drug | Mechanism of action |
|---|---|---|---|
| Acetylcholinesterase (AChE) | Breaks down acetylcholine, leading to cholinergic dysfunction in Alzheimer's disease | Donepezil, rivastigmine, galantamine | Reversible inhibition of AChE, increasing acetylcholine levels in the brain |
| Monoamine oxidase (MAO) | Breaks down monoamine neurotransmitters (dopamine, norepinephrine, serotonin), leading to neurodegeneration in Parkinson's disease | Selegiline, rasagiline | Selective and irreversible inhibition of MAO-B, reducing the breakdown of dopamine in the brain |
| Tyrosine hydroxylase (TH) | Involved in the production of dopamine, deficiency of TH leads to dopamine deficiency in Parkinson's disease | Levodopa | Precursor of dopamine that can cross the blood-brain barrier and increase dopamine levels in the brain |
| Catechol-O-methyltransferase (COMT) | Breaks down levodopa and dopamine, reducing their efficacy in Parkinson's disease | Entacapone, tolcapone | Inhibits COMT, increasing the bioavailability of levodopa and prolonging its effects |
| GABA aminotransferase (GABA-T) | Involved in the metabolism of GABA, deficiency of GABA-T leads to increased GABA levels in epilepsy | Vigabatrin | Irreversible inhibition of GABA-T, increasing GABA levels in the brain |

Then we have the cytochrome P450 enzymes and it metabolizes the toxin and drugs and you can actually be able to use this particular inhibitors. And it inhibits the cytokine production and down regulate the CYP2E1 expressions. Then we have the alcohol dehydrogenase and aldehyde dehydrogenase and that metabolizes the alcohol. You can

actually be able to use this particular inhibitor and it inhibits the ADH and activity causing the buildup of the acetaldehyde.

Then we have some of the enzyme which are being targeted in the treatment of the neurodegenerative diseases. So, we have the acetylcholinesterase, we have the monoamine oxidase, we have the tyrosine hydroxylase we have the Catecholine-O-methyltransferase and then we have the GABA aminotransferase.

And the all these enzymes are being inhibited by the some of these inhibitor molecules and the what the role is that they are actually going to this reverse the effect. And as a result, they are actually going to provide the relief into the disease, relief into the disease or they are actually going to reduce the symptoms.

Apart from these roles that enzymes are you know been used as a target or enzyme being targeted by the different types of drug molecules, enzymes can also be able to have the crucial role in drug metabolism. Because when the drug you are taking a drug, it is entering into the blood stream and from the blood stream it goes into the liver and from liver it goes to the all over body, right.

So, when it goes into the liver the primary function of the liver is that it actually going to reduce the level of toxicity of these drugs so that they can be able to get secreted out or excreted out from our body. So, there are enzyme which are even involved into the drug metabolisms.

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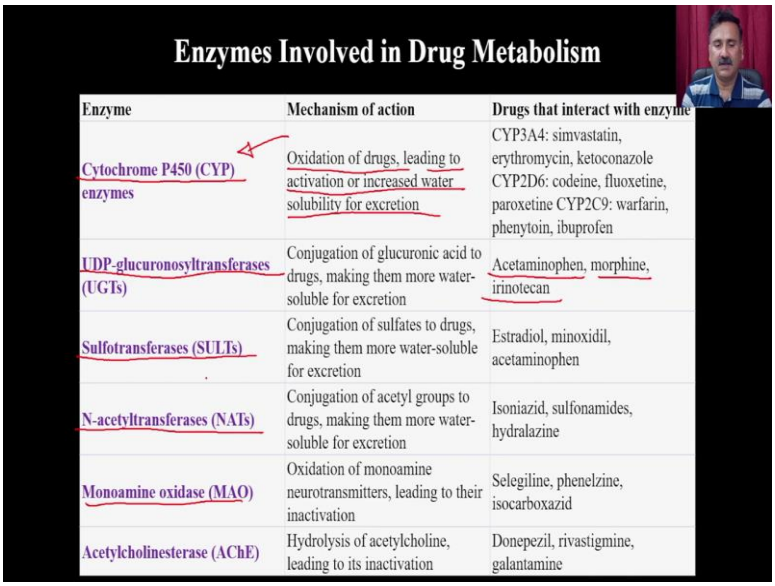


So, these are the some of the drugs which are involved in these are the some of the enzymes which are involved into the drug metabolism such as cytochrome P450, UDP-glucuronosyltransferase, sulfotransferase, N-acetyltransferase, monoamine oxidase and the acetylcholinesterase.

Cytochrome P450 it you know has some oxidation of the drugs leading to the activation or increase water solubility for the excretions. And these are the drugs that are actually been available to inhibit the activity of these enzyme. Because when they inhibit, they are actually going to have the higher level of drugs into the blood circulations.

Then we have the UDP, gluco UD UGTS and its conjugation of glucuronic acid to the drugs making them more water soluble for excretions and you can use the acetaminophen, morphine and this drug. Then these drug also. So, most of these inhibitors are inhibiting the drug metabolism in such a way that the level of drug is going to be more and as a result it is actually going to give you the relief.

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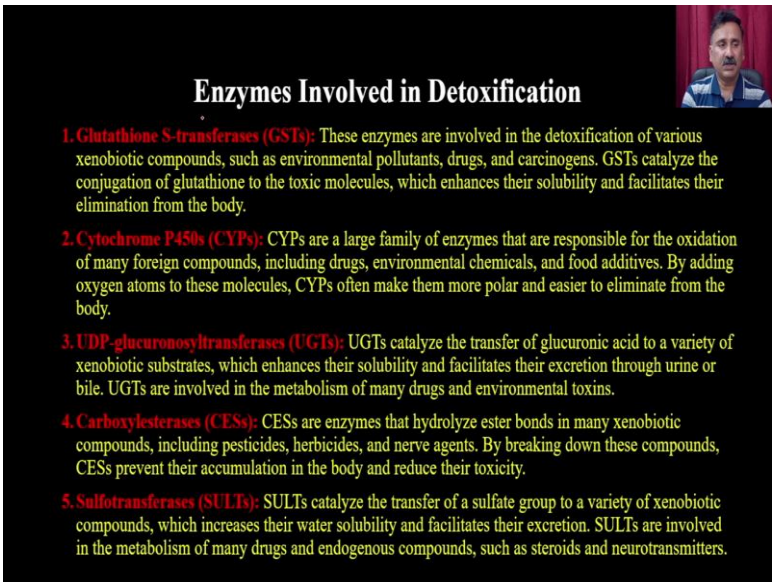


Apart from that there are enzyme which are involved into the detoxifications you can have the glutathione S-transferase you can have the cytochrome P450, you can have UDP glucuronosyltransferase, you have the carboxylesterases and then you have the sulfotransferases and these are the mechanism through which they are actually going to involved into the detoxifications.

So, this is all about the enzyme applications into the medical field. What we have discussed? We have discussed about the role of enzyme into the infectious diseases, we have discussed about the role of in the non-infectious diseases, we have discussed about how you can be able to develop the different types of drug molecules and that actually going to inhibit the different enzymes.

And that is how they are actually going to work as the you know the therapeutic molecules and that is how they are actually going to block the activity of these enzymes. And how you can be able to use these inhibitors to reverse the effects and that is how you can be able to cure the disease.

So, with this I would like to conclude my lecture here. In our subsequent lecture we are going to discuss more about the enzyme applications into the how you can be able to use the enzymes for modulating or controlling the activities of the environmental changes. So, with this I would like to conclude my lecture here.

Thank you.