Organic Chemistry in Biology and Drug Development
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Lecture – 60
Cholesterol Lowering Agents

Welcome back to this course on Organic Chemistry in Biology and Drug Development. In the first half of the lecture series, we have discussed the different biochemical processes where organic chemistry plays a dominant role. Then we started the next part of the course which is organic chemistry in drug development.

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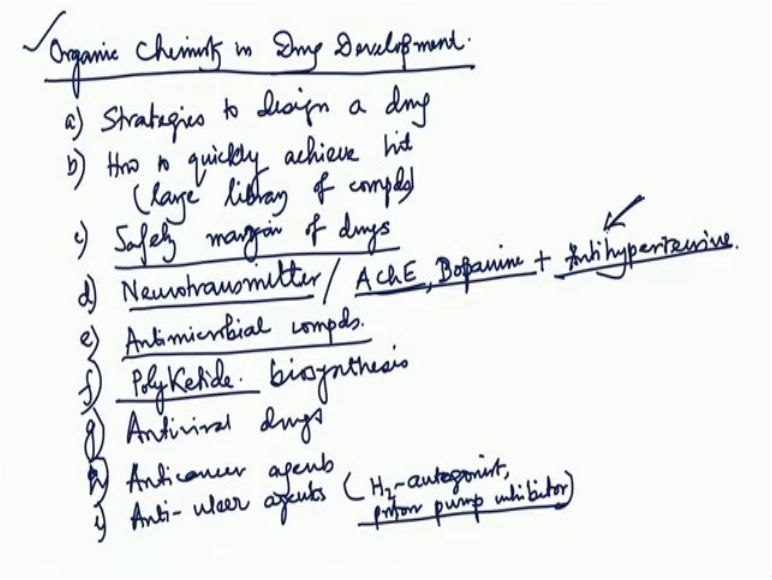

Basically this needs a very solid foundation of biochemistry knowledge. We are almost finishing this second part also. Few things are remaining that we are going to cover. But before that I want to just summarize what have been done till now in the area of drug development.

So, initially we discussed those strategies to develop and to design a drug. We have seen the importance of target identification, validation, and computational chemistry. Then we have seen the identification of a hit by in silico screening. After that, there are lead optimization preclinical studies and finally, the clinical studies involving human.

If everything goes properly the drug is approved by the food and drug administration authority in USA. It is considered as the best screening method. Because they approve the drugs only on the basis of all these parameters what we have discussed.

Now, then we went for that how to quickly achieve a hit. So, you have to have a large library of compounds and we discuss the combinatorial chemistry at that time. We have also discussed what is called the safety margin of drugs. Every drug has a side effect and also is toxic after certain amount of the dose. So, we have to be very careful about the therapeutic index of a drug because that is the margin which is allowed.

We have discussed the therapeutic index in terms of LD50/ED50. Now, ED50 means effective dose for 50 percent of the population and lethal dose means 50 percent of the population. Or it could be $TD_{50}/ED_{50.}$ TD_{50} is the therapeutic index. The higher the therapeutic index the safer is the drug.

Then we went for the different classes of drugs. Then we have discussed neurotransmitter, acetylcholine AChE acetylcholine, the dopamine. We discussed their production, their degradation and whatever effect they produce when binding to the receptor. This is neurotransmitter related in act brain dysfunctions.

We have discussed the antimicrobial compounds, antihypertensive drugs. We have studied the angiotensin converting enzyme and the hormone angiotensin 2. This is the hormone related hypertension.

Then we discussed antimicrobial compounds in detail. We have discussed the penicillin, the sulfonamides, the tetracycline, the erythromycin. Then we discussed the polyketides in connection with the erythromycin polyketide biosynthesis. A majority of the drugs are natural products and a majority of them are polyketide.

Then we discussed the design strategy of antiviral drugs. Then we went to anticancer agents. In the last session, we discussed the antiulcer agents. Antiulcer agents are either H_2 antagonists or proton pump inhibitor. We have discussed the chemistry of ranitidine and also the chemistry of omeprazole which is acting as proton pump inhibitor.

So, we will now move on to another aspect which is also related to hypertension. Hypertension is basically the blood pressure higher than the normal level. Now

hypertension can be due to several factors. One of them is over production of angiotensin 2.

Another was modulating the calcium channel blocker. Sometime beta blocker means these beta adrenergic receptors can control the flow of blood towards the heart and outside. Apart from these another factor which is also important is that person suffering from having a high cholesterol level in their blood. That cholesterol after all has a huge hydrophobic skeleton.

Solubility of cholesterol in biological solvent system is not very high. This cholesterol is transmitted from one place to another by the low density lipoprotein LDL and the high density lipoprotein HDL. These carry the cholesterol from one place to another. If the cholesterol limit is crossed over the normal limit there is cholesterol deposition in the arteries.

So, your passage inside the arteries becomes narrow. Flow of blood will have lot of hindrance. But, a narrow passage will result in a back pressure. It is another cause of having high hypertension

If your artery is already having a deposition of cholesterol you cannot remove that unless you have a surgery. Otherwise the cholesterol that has been deposited inside the artery will acts as a vasoconstriction. So, the pressure goes up. Sometimes this deposition can come. Like, if you boil water in a kettle for a long period of time you will find a layer of calcium carbonate at the bottom. Sometimes chunks of calcium carbonate can come up in the water when you boil. This deposit is containing other molecules, but cholesterol is the predominant part. So, if these chunks come up in the bloodstream that can block the valve associated with the heart. Or it can go and block the nerves.

So, if that happens then in the first case you have stroke and in the second case what you call cerebral attack. Heart attack versus cerebral attack is the cause of the cholesterol deposition in your artery.

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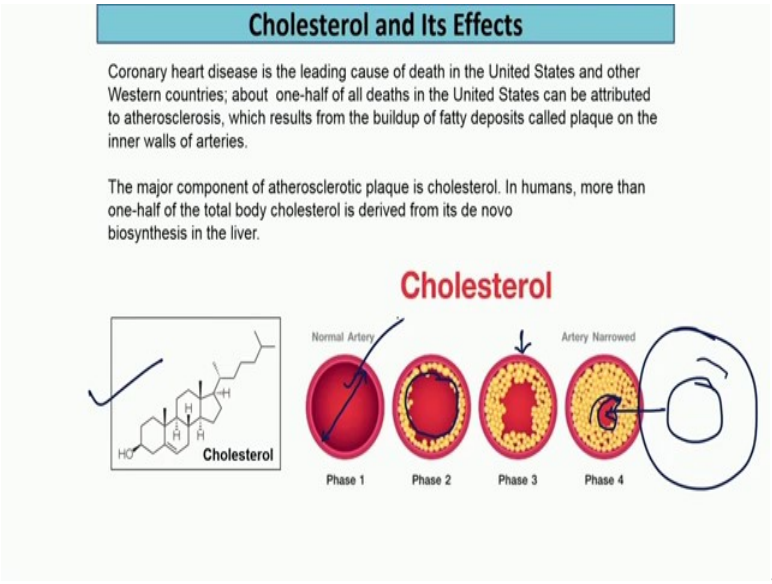

This is the structure of cholesterol in the picture. Now you see this is the normal artery, there is no deposition and that is the total inside diameter of the artery through which blood can flow. If you have higher cholesterol level in blood some insoluble cholesterol will come out and then they will deposit inside the wall of the artery.

If you have more deposition more deposition then in extreme case the passage becomes so narrow. So, you have the risk of having a heart attack or a cerebral attack. Now, if you have this type of scenario I will say about 50 percent blockage or 60 percent. Here the blockage is more than 80 percent ok. This is still ok but one has to be extremely careful that if the chunks come out then it can block the valve or the nerve endings.

I will device a molecule which will dissolve the cholesterol from the artery. But, that is very difficult because one which has already forming a crust inside the blood vessel. Here remedy is surgery that creates a bypass. Or sometimes they dilate the blood vessels which are called balloon angioplasty.

Whatever narrow is there that becomes bigger by putting pressure. So, it is the decision of a doctor. How one can maintain the cholesterol at the normal level. If you keep the cholesterol at the normal level your artery will be similar to this.

How will you design this cholesterol lowering agent?

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Antihypercholesterolemic drugs will lower the higher value of cholesterol to the normal level.

Now cholesterol can come into our bloodstream in two ways. One is metabolic that we make our own cholesterol i.e. the biosynthesis of cholesterol and the other is from the food. Animal origin can also contribute to cholesterol. It can contribute to proteins which carry the cholesterol towards from one place to another.

About 80 percent of the cholesterol of human body is biosynthesized inside the body. About 20 percent we take from the food. So, food is not the ultimate culprit. The culprit is the metabolic disorder that is present in the body.

Antihypercholesterolemic drugs can be designed by targeting the biosynthesis of cholesterol. What are the steps for biosynthesis of cholesterol? Around 30 enzymes are involved in the biosynthesis of cholesterol.

Cholesterol is biosynthesized from the acetyl coenzyme A. Acetyl coenzyme is also starting material for polyketides or the fatty acid biosynthesis. I can stop the biosynthesis of cholesterol by having an enzyme inhibitor which is an irreversible inhibitor.

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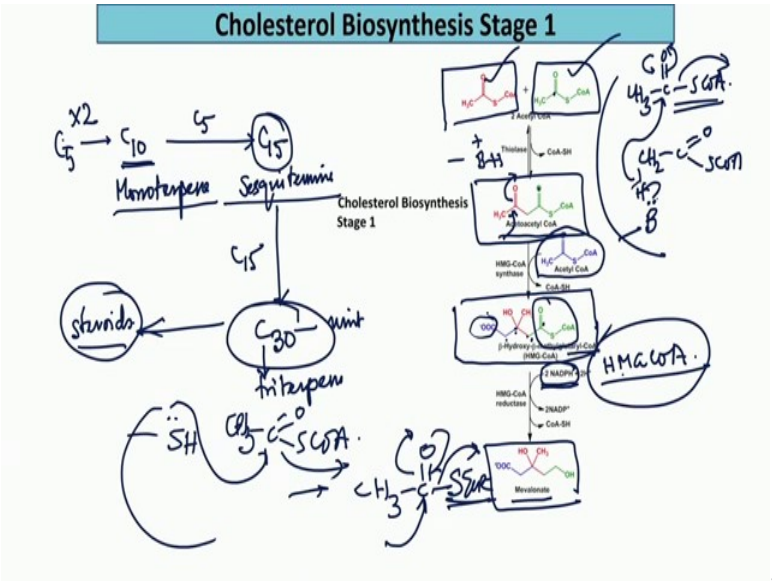

Cholesterol is also an obligatory ingredient for other body functions. It is present in the cell membrane, lipid bilayer. Cholesterol is the precursor for making different hormones, like estrogen, androgen. So, you cannot completely shut down the level of cholesterol. We can design a reversible inhibitor to reduce the efficiency of the cholesterol making enzymes. We have to put at an optimum level and we cannot make it very low because there will be starvation of this cholesterol which is required for other body functions.

What are the reactions involved in cholesterol biosynthesis. Now there is another class of compounds which is present in plants and they are called terpenes which are also very similar to the cholesterol. The cholesterol has a fully dearomatized phenanthrene ring attached to a five membered ring which is called cyclo penteno phenanthrene skeleton.

On the other hand, in perhydro phenanthrene skeleton every double bond is hydrogenated and terpene is usually 5 6 membered rings fused together. So, that is the difference between terpene and cholesterol. The biosynthesis is very similar but at some point they branch out.

The precursors for biosynthesis of terpene and cholesterol are same.

There are 30 carbons in cholesterol. In case of triterpene, there are 30 carbons. So, basically it starts with a C5 unit which first goes to a C10 unit. This is the called what is called a monoterpene. Then the C10 goes to C15. So, that means, first two C5 units

condense with each other to give C10. Then the C10 condenses with a C5 unit. Sesquiterpene is the 15 carbon containing compound and then 2 C 15 combines and you get C30.

This is either could be triterpene because from this C30 the triterpene could be obtained. Then that also gives rise to steroids. So, the branching point is here. From C30 you branch out either to the terpenes or to the steroids. The names are actually not in terms of terpene because steroid is only a name given to C30 carbon containing compounds.

But, there may be some cases where the carbon may be less than 30. I should also warn you that because cholesterol has a side chain here. So, it may be totally oxidized by the system and it ultimately becomes a OH or ketone. Then you do not have 30 carbons. But, you see the precursor is 30 carbon.

So, steroids are either 30 carbon containing compounds or it could be compounds which are derived from 30 carbon containing compounds where the side chain is oxidized is broken down or degraded. So, you should not go away with a message that all steroids have thirty carbons. That is not true. Cholesterol has 30 because it is almost the immediate precursor of C30. Now let us see reactions involved.

I told you the precursor is acetyl coenzyme A. Now this chemistry will be easier to understand because we have already gone through the chemistry of the polyketide. In polyketide chemistry, one acetyl coenzyme A and a malonyl coenzyme A combine to form the acetoacetyl coenzyme A. But, here two acetyl coenzyme A combines directly. It is not a decarboxylative pathway. Basically this is an ester and ultimately you need a base.

So, this forms the enolate and the enolate then attacks the carbonyl. So, you have CH_3 then SCoA which is usually very labile. But the other part it is a hydrogen CH_2 CO SCoA. Now, you have a histidine in the enzyme system. So, that abstracts this hydrogen. So, this comes and attacks to the oxygen, it comes back and SCoA goes out.

However the actual mechanism is more complicated. The first attack on an acetyl CoA takes place by a cysteine sulfur. That means, the enzyme is like this SH. So, that first displaces the thioester. This acetyl CoA is first displaced. So, you get CH_3CO and I can write this S enzyme. Instead of thio, the coenzyme is sulfur containing. Then the other

part is enolized by an imidazole base. That comes and attacks this compound. That is the precise mechanism. But in just for simplicity you can say that two molecules of acetyl CoA condenses to form acetoacetyl CoA.

Now, in case of polyketide this gets either reduced or this will be extended another malonyl CoA which is the extension unit. In this case, there is a third reaction. This first reaction is occurring between two acetyl coenzyme A and the second reaction is another acetyl coenzyme A reacts with this carbonyl and makes it a OH. Here we have to abstract this hydrogen. Imidazole pulls up the hydrogen.

Imidazole means it comes from a histidine and you have to have a hydrogen donor. Because, you know that addition to the carbonyl is catalyzed by acid. So, a hydrogen donor will be better and that will be in the form of a BH^+. Imidazolium cation could be a protonated base. That gives the hydrogen to the developing negative charge on the oxygen. Now alongside this you also have a hydrolysis of this acetyl CoA and if acetyl CoA is hydrolyzed you will get acetic acid.

So, basically this was earlier acetyl CoA. Here CoA leaves and you get the free acid. That is called β-hydroxy- beta methyl-glutaryl Coenzyme A and in abbreviation it is called HMG CoA.

This is basically a expulsion of one CoA. In the next step, you have a nucleophilic addition to the beta carbonyl. That is associated with the hydrolysis of the CoA. So, that one end you have a CO minus and the other end you have CoA thioester. The thioester is now reduced by a NADPH mediated enzyme. So, you will get a compound where the ester is reduced to the alcohol. It is a reduction from acid to alcohol. So, it first goes to the aldehyde and then goes to the alcohol. They have found that it is a NADPH mediated hydrolysis. But two molecules of NADPH will be required because you are doing reductions from acid to the aldehyde and aldehyde to the alcohol.

This compound is called mevalonic acid in the biological system because it is present in anion. So, that will be called mevalonate.

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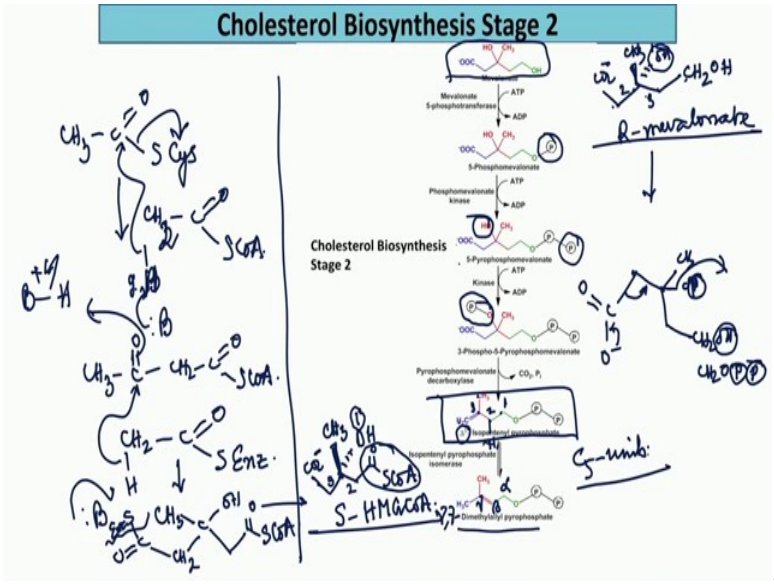

In the first condensation reaction, this cysteine displaces the coenzyme A. This is hydrogen and the histidine is base. So, base abstracts the hydrogen and it attacks. Ultimately the cysteine leaves. So, you get the first compound acetoacetyl coenzyme A.

In the second reaction you have again a displacement of the acetyl. There is another acetyl CoA which has to react with the carbonyl. The enzyme has displaced the CoA. Again the imidazole goes there and this abstracts a proton.

So, that will give you hydroxymethyl. You write a carbon with CH_3 ,OH and then you have on one side CHC_2, then CoS enzyme because still that is attached to enzyme. On the other hand, you have CH_2 COS CoA. Now water comes and hydrolyzes this. So, you get this HMGCoA.

Now, interestingly the configuration is also important because it is a stereo specific reaction. You don't get because you are developing a chiral center here. It will be S-HMG CoA.

The next step is reduction of this thioester into the alcohol. So, that is given here although the stereochemistry is not given. This is also interesting that the stereochemistry remains the same because you are not touching the stereogenic center in this reaction. The NADPH mediated reduction is basically touching the thioester.

It will be R mevalonate but HMG CoA has opposite configuration. It is just the priority sequence order that has got changed.

So, R mevalonate is formed. This is C5 unit. How the C5 unit is formed. You can form the C5 unit. You do a decarboxylative elimination like CO_2 minus and then make OH as a good leaving group. You have to do phosphorylation because OH is a bad leaving group.

It has been found this OH is diphosphorylated. At first, the elimination takes place by putting a phosphate on this primary alcohol and then another phosphate. Diphosphorylation are all ATP mediated reaction.. Now this undergoes again phosphorylation. So, you have the leaving group O phosphate and then this decarboxylation is taking place. You will get a isopentenyl pyrophosphate.

Isopentenyl pyrophosphate can equilibrate between two tautomeric forms. This hydrogen can undergo 1,3-shift and you get the double bond inside. So, this is dimethyl allyl pyrophosphate. Involvements of condensation between the three acetate units lead to the HMG CoA.

And, then HMGCoA undergoes phosphorylation, and decarboxylation and ultimately you get a mevalonic acid which is in the R configuration and then through the decarboxylation you get isopentenyl pyrophosphate which is in equilibrium with gamma dimethyl pyrophosphate. So, these are all C5 units.

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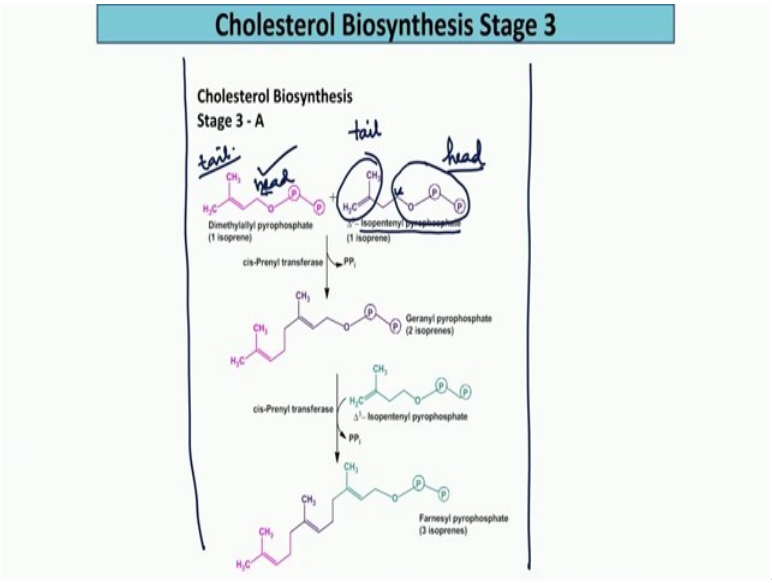

This is γ,γ-dimethyl allyl pyrophosphate and this is isopentenyl pyrophosphate. You have to this part where the polar moiety is present that is called the head group and where the nonpolar or the lipophilic part is present that is called the tail. So, this isopentenyl pyrophosphate has a head group and a tail end.

So, for this also the head is here and the tail is here. Tail carbon is these methyls and that the head carbons is this allyl. In some books it is written in the opposite way. But usually, in a fatty acid the head group is the acid group and the fatty chain end group is the tail group.

Now there will be a condensation between these two. In the next session, we will start from here. So, we have discussed the formation of the two C5 units which are in equilibrium with each other. Remember, these are the two most important units - one is γ,γ-dimethyl allyl pyrophosphate, another is isopentenyl pyrophosphate which comes from the R mevalonate.

Thank you.