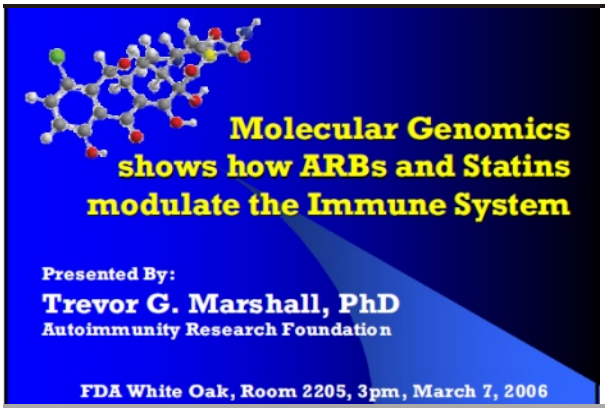
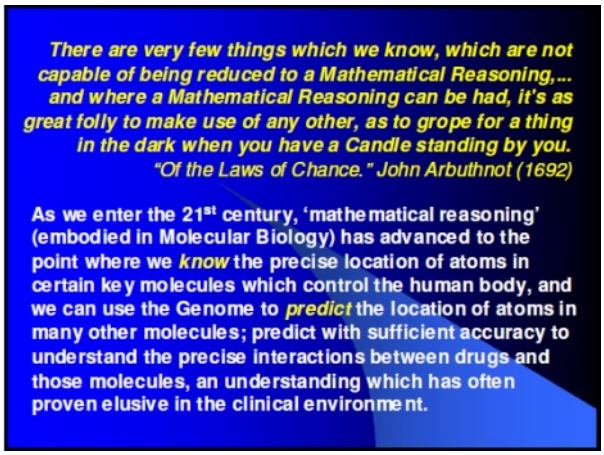


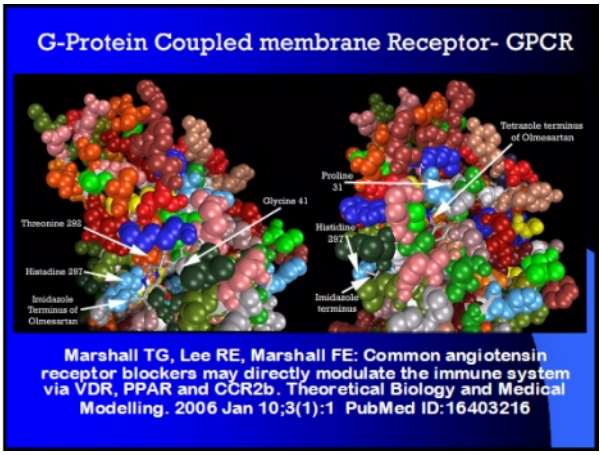
Molecular genomics offers new insight into the exact mechanism of action of common drugs: ARBs, statins and corticosteroids

Trevor G. Marshall, PhD
Visiting Professor Lecture Series,
Center for Drug Evaluation and Research,
a division of the FDA
Bethesda, MD
March 7, 2006
http://www.youtube.com/watch?v=IsHWoRpkTw0

Autoimmunity Research Foundation
A metagenomic understanding of chronic disease
THOUSAND OAKS, CA, USA



"Molecular genomics... is a complementary technology, it offers alternative insights.



[00:00:22]
Mathematical Reasoning and Molecular Genomics

While preparing these slides, I recalled this quotation (1692) from John Arbuthnot, who was one of the first people to actually use epidemiology and statistics in the study of disease.

"There are very few things which we know, which are not capable of being reduced to a Mathematical Reasoning … and where a Mathematical Reasoning can be had, it is as great folly to make use of any other, as to grope for a thing in the dark when you have a Candle standing by you."

I just thought that seemed to encapsulate what molecular genomics offers to clinical medicine at this point in time. It is a complementary technology, it offers alternative insights, and that is what I am going to be talking about today.

Because as we enter the twenty-first century, 'mathematical reasoning,' which is specifically embodied in molecular biology, has advanced to the point where we know the precise location of atoms in certain key molecules which control the human body, and we can use the genome to predict the location of atoms in many other molecules, and predict with sufficient accuracy to understand the precise interactions between drugs and those molecules; an understanding which has often proven elusive in the clinical environment.

That is, in fact, exactly what we are talking about here. You will all be aware of the tremendous amount of research that has been expended on trying to figure out how ARBs differ from Statins, and differ from ACE inhibitors. The molecular genomics can help us understand what to look for when we go back into the clinical environment.

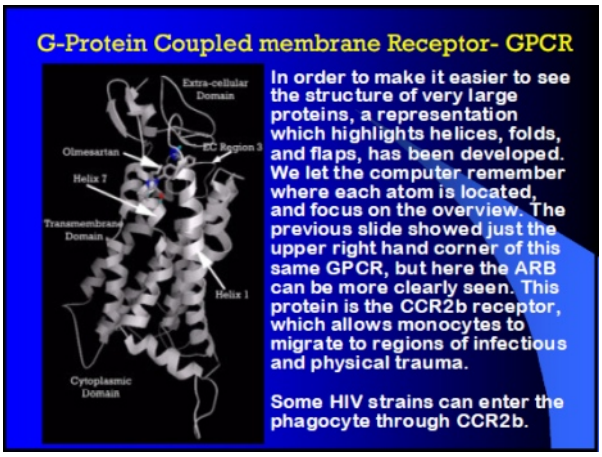
[00:02:13]
G-Protein Coupled membrane Receptor–GPCR

So we are talking about atoms. Well, there are lots of atoms in the human body, far too many for us to consider individually.

This is a picture from one of the figures from our recent paper, "Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b." Those are all molecules in the human body that have specific functions.

This particular picture might look rather pretty, but it is not very useful for its major purpose. Its major purpose is to show that this ARB, here, is docking—has a strong affinity for—the receptor. There is the rear of the ARB molecule and here is the front of the ARB molecule.

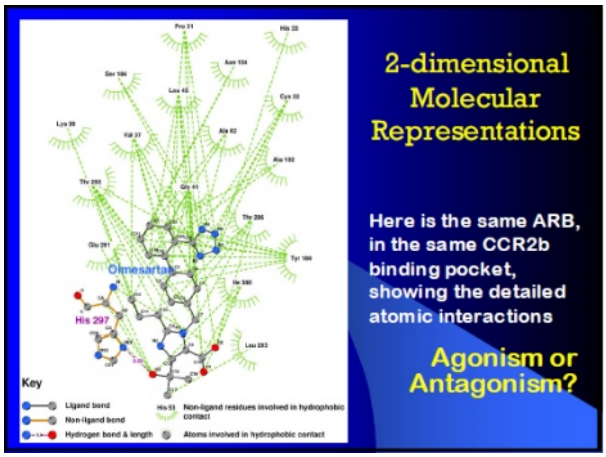
But using a representation like that is not very helpful in terms of trying to understand how these molecules actually work.



[00:01:03]
G-Protein Coupled membrane Receptor–GPCR

So in order to make it easier to understand the structure of very large proteins, a representation which highlights helices, folds and flaps has been developed. We let the computer remember where each atom is located, and focus on the overview.

The previous slide showed just the upper right hand corner of this same GPCR, but here, the ARB and the binding pockets can be far more clearly seen. This protein is the CCR2b receptor, which allows monocytes to migrate to regions of infectious and physical trauma. Also some HIV strains enter the phagocyte through CCR2b. But they are very important molecules.

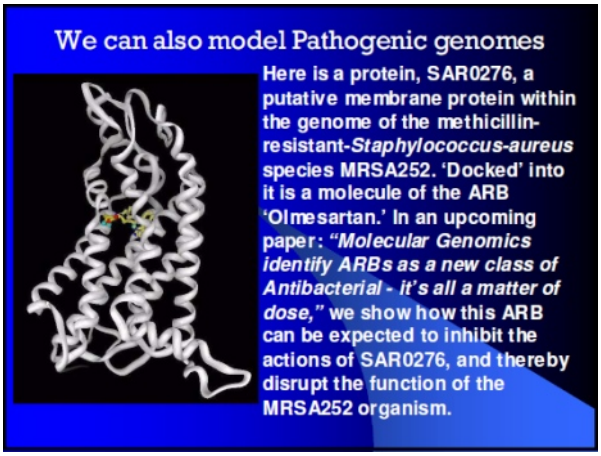


[00:03:58]
Two dimensional Molecular Representations

We can also produce two-dimensional molecular representations, which are extremely useful when we are trying to figure out whether we are looking at an agonist or an antagonist—whether the drug is acting to enhance the operation of the receptor, or to block operation of the receptor.

Here is the same ARB in the same CCR2b binding pocket, but now you have got the details of atomic interactions.You have got each of the residues, each of the amino acids in the receptor, and specific lines showing which of the ligands or which of the ARB atoms are within bonding distance, or certainly a Van der Wiel system.

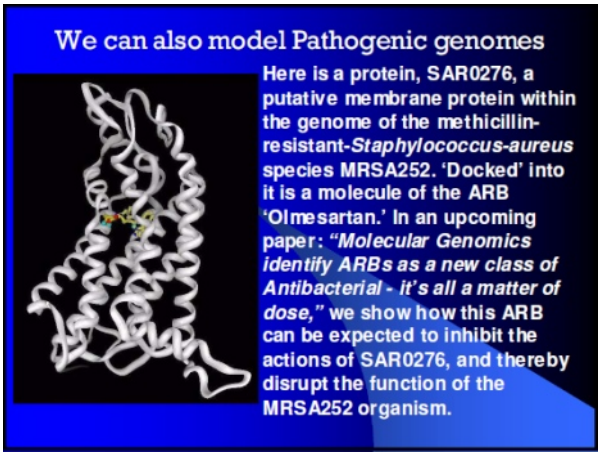
And here is the hydrogen bond. The hydrogen bonds are very important because they tend to be quite a lot stronger and they orient the molecules in the receptor. But in general, you only go to these representations when you are going for extreme detail. It is far too complex otherwise.



[00:05:08]
We can also model Pathogenic genomes

Now, we can also model pathogenic genomes. Here is a protein, protein SAR0276, which is a putative membrane protein within the genome of the methicillin-resistant-Staphylococcus-aureus species MRSA252 – Staphylococcus protein.

"Docked" into it (or mated with it or bound to it) is a molecule of the ARB "Olmesartan".



In an upcoming paper of which we have submitted, "Molecular Genomics Identify ARBs as a new class of Antibacterial—it's all a matter of dose," we show how this ARB can be expected to inhibit the actions of the protein, and thereby disrupt the function of the MRSA252 organism.

In this particular case, the way we came across the protein and realized that it was actually a GPCR family was by using standard genomic search techniques. Beside the molecule was CCR2b, which is the molecule I showed you a little while ago, the one that is prevalent on myocytes and forces the myocytes to migrate to areas of trauma. This particular protein is in the MRSA genome, and its function? We do not know. But if it is ever expressed by the organism, and if the ARB is present in the blood stream of the individual, the ARB will go after that particular protein and bind into it quite firmly.

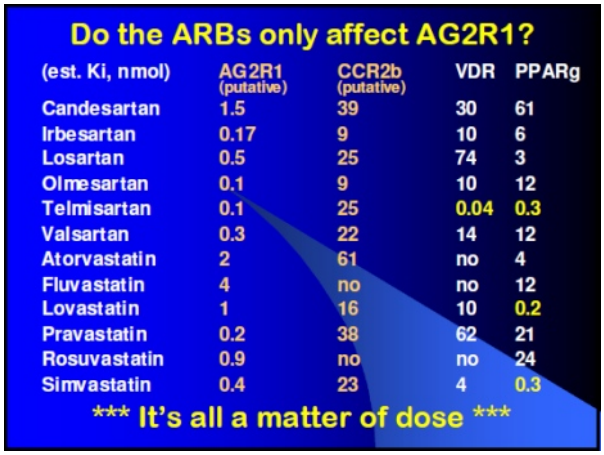
"It was very interesting as to why bacteria would have an affinity for ARBs.

[00:06:59]
[Discussion]

Why do I have this here? Well, it turns out this whole search, that led to the presentation here today, started off with some papers back in the early nineties, where biochemists that were working on the development of ARBs found that unless they applied a bactericide to kill any bacteria in the tissue samples, the radiolabelled ARB was taken up by the bacterial organism and not by the tissue that was under test.

At the time I had no idea what was going on or why this would be the case, and the biochemists did not care about it. They just made sure they killed the bacteria in any tissue samples before they did their testing.

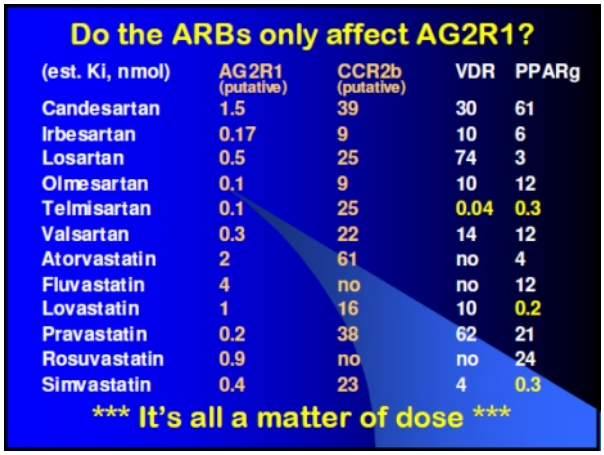
But it was very interesting as to why bacteria would have an affinity for ARBs. That is really what started me off on this search, and that is why it is here, at this point in time.

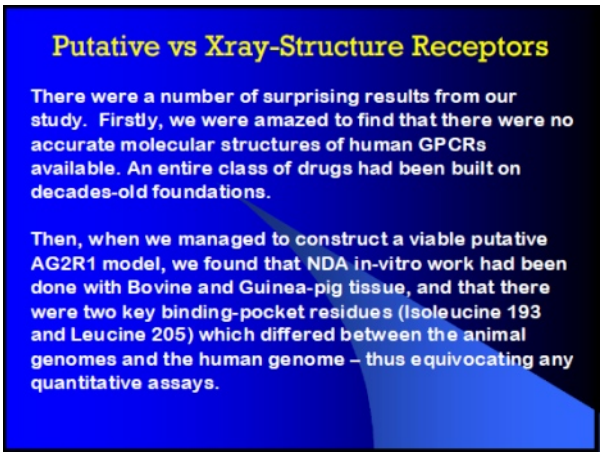


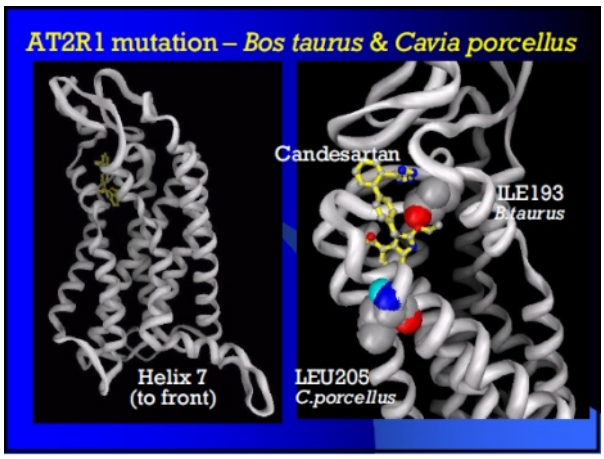
[00:08:04]
Do ARBs only affect AG2R1?

Now, what we have done is a very large computer search, using a computer service, running Linux and some software which automatically docks ligands, or drugs, into proteins. We have taken known proteins and some hypothetical proteins where we do not have an x-rayed structure, so we cannot be precisely sure that these are the correct shape (but we think they are), and then we have taken known proteins VDR and PPARg which have actually been photographed with x-ray technique—in order to find out exactly where the atoms are. And we have matched up some drugs with the receptors.

As you can see from this table, most of the ARBs and statins have some affinity for angiotensin 2 receptor which is a GPCR (that is a receptor like we were looking at earlier, a membrane receptor), CCR2b (which is a putative model again), and VDR and PPARg, which are both nuclear receptors.







There are some that do not dock, and there are some that dock with quite high affinity.

Losartan, for example, would not normally inhibit the function of the VDR at the concentrations that the drug is normally administered. But other drugs such as Telmisartan in the VDR, clearly, significantly affect the operation of both VDR and PPARg at normal concentrations.

This graph is in nanomoles. So with a 25 milligram daily dose of Olmesartan, for example, it will create a bloodstream concentration that will affect up to around 10 nanomolar affinity; and certainly at the 0.04 and 0.3 nanomolar, there will be very great interaction.

[00:10:30]

Putative versus X-ray Structure Receptors

Now, there were a number of surprising results from our study. Firstly, we were amazed to find that there were no accurate molecular structures of the human membrane receptors, the human GPCR's, available.

The entire class of drugs, the ARBs, had been built on decades-old foundations, totally in vitro work.

Then, when we managed to construct a viable putative model, because we had to go back to fundamentals and say, "Well, can we construct a model for the angiotensin 2 receptor that makes sense, that has the conserved regions in the right spot, that binds the drugs that we know are binding to it, that binds them with correct affinity?" That is what we call a putative model. Not verified with x-ray, but it gives us something to work from.

At that point we found that the NDA, the new drugs application's in vitro work, had been done with Bovine and Guinea pig tissue, and that there were two key binding-pocket residues (Isoleucine 193 and Leucine 205) which differed between the animal genomes and the human genome, thus equivocating (making uncertain) quantitative assays.

[00:11:54]

AT2R1 mutation—Bos taurus & Cavia porcellus

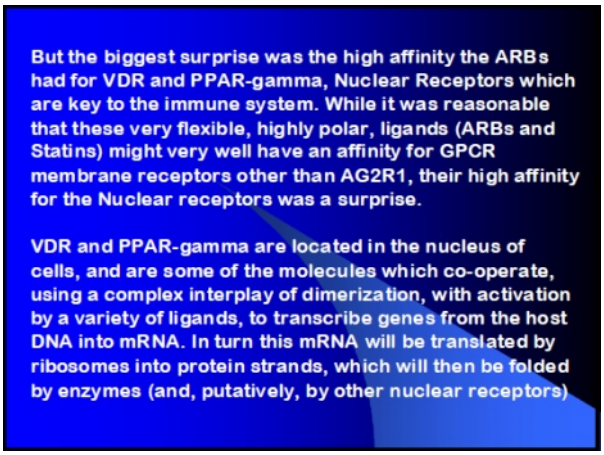
I have highlighted the two residues that are mutated, here. This residue is mutated in Bos Taurus, and this residue is mutated in Cavia porcellus. You can see I have got a Candesartan, an ARB, bound into the binding pocket, and there is actually an oxygen on the Candesartan that is very tightly bound into the Isoleucine 193 of Bos Taurus.

One of the things that we found in our study was that we could not (as accurately as we expected) match up the expectations for the binding affinity of Candesartan that were listed in the NDA with the binding affinity that we were simulating in our receptor. Then when we had a look and realized that the NDA had been done with Bos taurus, a protein from the animal genome. We suddenly realized

that there is a huge difference in affinity at this point; and that tended to make us feel a little bit more comfortable with our model for the angiotensin receptor.

Here is the entire picture of the GPCR, and you can see the binding pocket is up here, behind helix 6, and between helices 5, 4, and 6. The Candesartan is bound up there.

I might add that this is a membrane protein so most of the central region of the protein is within the membrane, the region at the bottom is in the cytoplasm, and the region at the top is extra-cellular.... This is the trans-membrane region, this is the extra-cellular region, and this is the intra-cellular and signaling region.



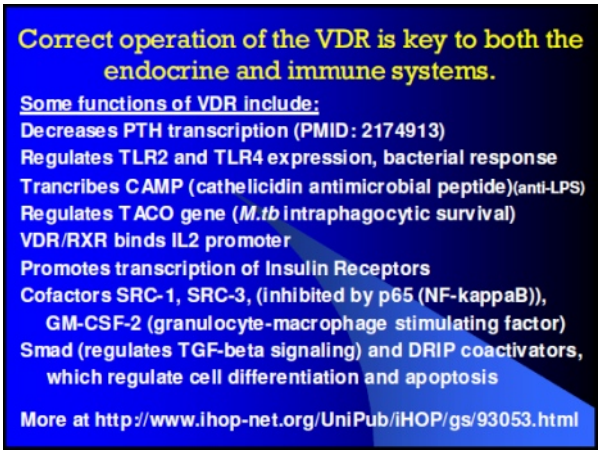
[00:14:15]

Affinity of ARBs for nuclear receptors

But the biggest surprise we had was the extremely high affinity which the ARBs had for VDR and PPAR-gamma, which are nuclear receptors. These are in the nucleus, not on the membrane of the phagocyte, but in the nucleus of the phagocyte. And they are key to the operation of the immune system. So while it was reasonable that these highly flexible, polar ligands, the ARBs and statins (ligand is a technical term for drug) might well have a good affinity for other GPCRs (membrane proteins) other than angiotensin 2 type 1 receptor. We never expected them to have such a high affinity for the nuclear receptors.

VDR and PPAR-gamma are located in the nucleus of cells, and are some of the molecules which cooperate, using a complex interplay of dimerization (dimerization is where proteins bind to each other to form multiple complexes called dimers), with activation by a variety of ligands which transcribe genes from the host DNA into messenger-RNA. In turn, this RNA will be translated by the ribosomes into long protein strands, and they are then folded into the final shape—for example SPCA, SPCR shape. There are lots of enzymes, lots of folds. They are folded by obviously electrostatic forces, but also by enzymes, and there is some feeling that other nuclear receptors are involved in some protein folding as well.

But these are at the very heart of the genome. All of the proteins produced by the cell come from this DNA transcription process.



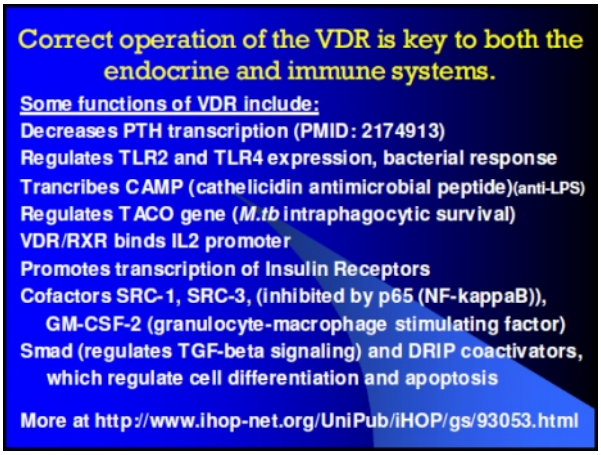
[00:16:24]

Correct operation of the VDR is key to both the endocrine and immune systems

VDR is the first one we will look at. The correct operation of the VDR is key to both the endocrine and the immune system.

Some functions of the VDR include:

- Decreased parathyroid hormone transcription. It lowers, high levels of VDR generally correlate with low levels of PTH, because it decreases the transcription of PTH.
- It regulates the Toll-like receptor 2 (TLR-2) and Toll-like receptor 4 (TLR-4) expression. These are receptors which are



on phagocytes, and they are part of the innate immune response. In fact, they are key to the innate immune response. VDR regulates them and consequently regulates the response of the body to bacteria.

It transcribes CAMP (cathelicidin antimicrobial peptides) and that is an endogenous antibiotic that the body makes, which attacks lipopolysaccharides on Gram-negative bacteria. There are a number of endogenous antibiotics, that is one of them. We know for certain that that is transcribed by the VDR.

It regulates the TACO gene, and the TACO gene is associated with Mycobacterium tuberculosis survival intraphagocytic—in other words, how the Mycobacterium tuberculosis, when it has invaded the cell, manages to survive phagocytosis. The VDR regulates the TACO gene.

It binds the interleukin-2 promoter, and therefore transcribes interleukin-2, another key immune cytokine.

It also promotes transcription of insulin receptors. It transcribes the DNA into RNA so that insulin receptors can be made.

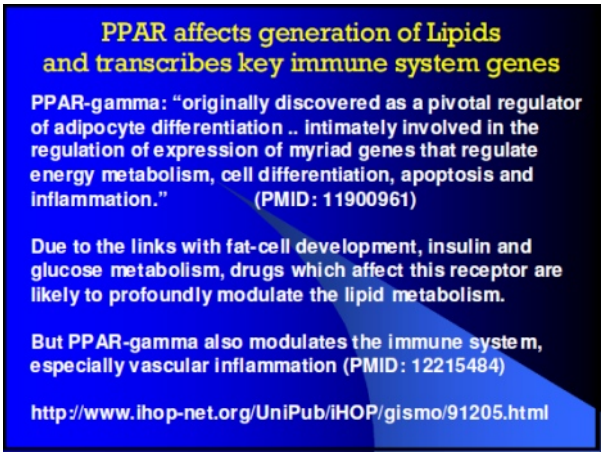
It interacts with cofactors SRC1 and SRC3, SRC steroid-receptor cofactors, which are inhibited by P65, which is half of the nuclear factor-kappaB. Again, immune system.

And we know it is associated also with the granulocyte-macrophage stimulating factor, another key immune system function.

It regulates TGF-beta signaling, and DRIP coactivators (DRIP is D receptor-interacting proteins), all of which regulate cell differentiation and apoptosis. There is a url of a search engine which will specifically search for citations on the VDR, if any of you are interested in looking further. (http://www.ihop-net.org)

> "There is so much [PubMed] activity in molecular genomics at the moment, looking at the VDR.

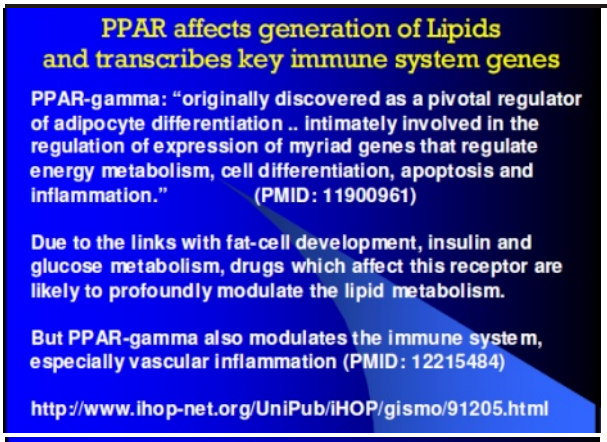
There is so much activity in molecular genomics at the moment, looking at the VDR. We published a paper not too long ago, and recently I wanted to look it up on PubMed... and rather than type in the full name and the author, the normal way, I just typed in the VDR characters and let the VDR carry through. I thought, "Well, it will be somewhere on the first page." No way! There have been forty papers published on VDR since ours in mid-January. That is a rate of about one a day. And half of those are on the immune system, and the importance of this receptor to the immune system.



[00:20:08]

PPAR affects generation of Lipids and transcribes key immune system genes

PPAR is another receptor. There are two forms (Peroxysome Proliferator Activation Receptor is the full acronym) but PPAR affects the generation of lipids, and it also transcribes key immune system genes.

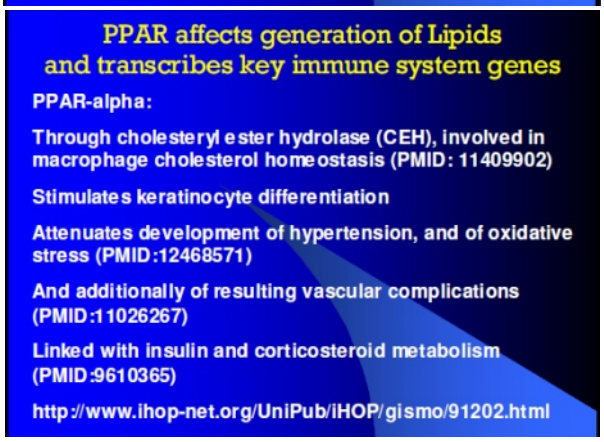


PPAR-gamma

And here is a point which I thought was rather good from one of the papers that I reviewed. PPAR-gamma was "originally discovered as a pivotal regulator of adipocyte differentiation," but it is "intimately involved in the regulation of expression of a myriad of genes, that regulate energy metabolism, cell differentiation, apoptosis and inflammation."

Due to the links with fat-cell development, insulin and glucose metabolism, drugs which affect PPAR-gamma are likely to profoundly modulate the lipid metabolism.

PPAR-gamma also modulates the immune system, especially vascular inflammation.

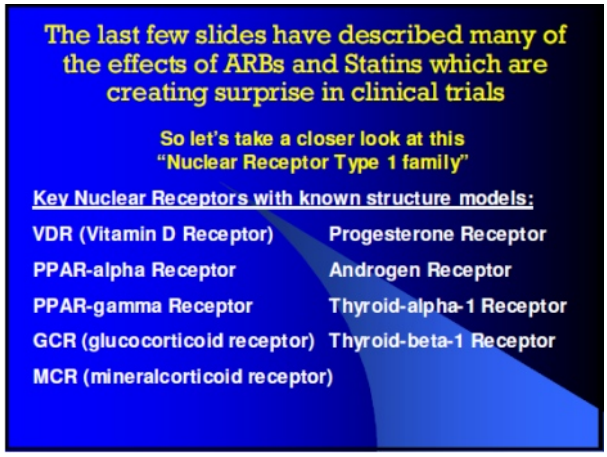


PPAR-alpha

The other receptor that we looked at initially was PPAR-alpha, which is involved in mediating cholesteryl ester hydrolase (CEH), which is part of the macrophage cholesterol homeostasis.

It also stimulates keratinocyte differentiation, and attenuates development of hypertension and of oxidative stress.

It additionally attenuates vascular complications, and it is linked with insulin and the corticosteroid metabolism. Again, a key receptor for the immune system.

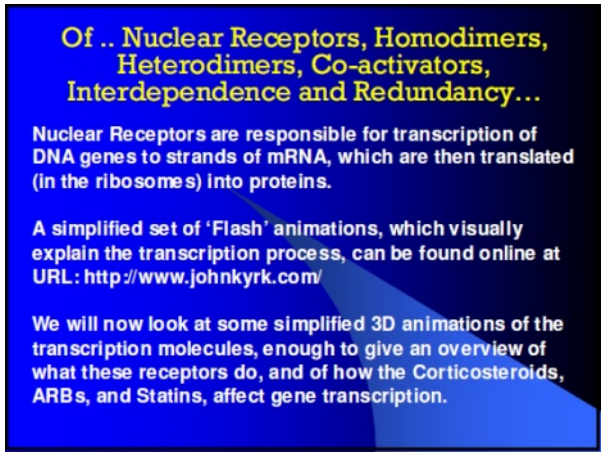


[00:21:56]

The Nuclear Receptor Type 1 family

The last few slides have described many of the effects of ARBs and Statins which are currently creating surprise in clinical trials. ARBs and Statins affect, for example, diabetes and certainly the complications of diabetes, and also artherosclerosis.

So we decided to take the key nuclear receptors which have got known structures for them, where we could be quite certain that we were dealing a real molecule and not with ones that we had derived and tested. They turned out to be the VDR, the PPAR-alpha, the PPAR-gamma, the GCR (glucocorticoid receptor), the MCR (mineralcorticoid receptor), Progesterone Receptor, Androgen Receptor, and Thyroid-alpha-1 and Thyroid-beta-1. There are four thyroid receptors, and we just selected those two because they were available as x-ray models.



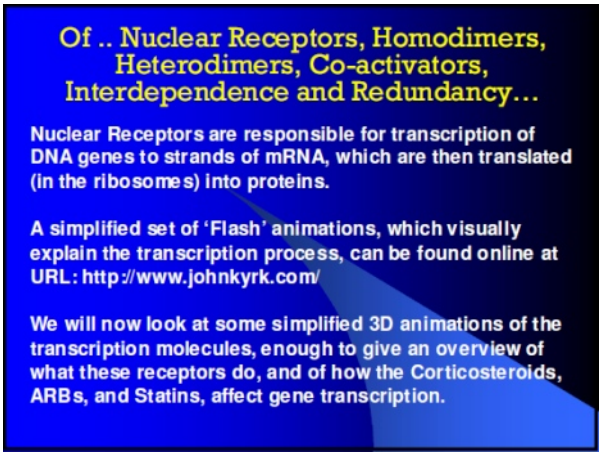
[00:23:05]

Nuclear receptors: transcription of DNA genes

Now what do these nuclear receptors do?

Well, they join together in heterodimers, or they couple within the same receptor as homodimers. They couple with co-activators, they are very interdependent, and they are very redundant.

For example, if you knock out the Beta-1 Thyroid Receptor from mice, then the mice end up deaf, but everything else seems to work correctly. So there is a lot of redundancy. However, if you knock out the GCR, the glucocorticoid receptor [NR3C1: nuclear

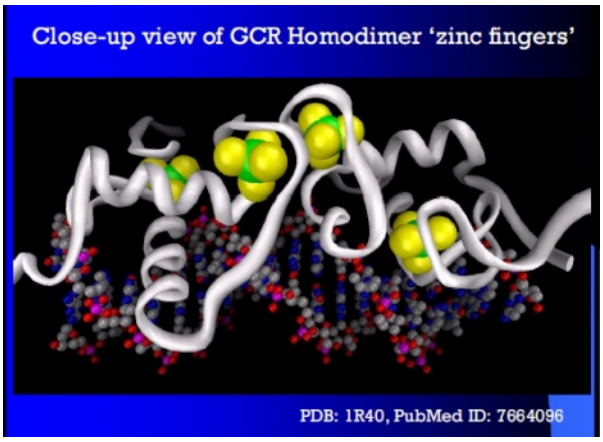


receptor subfamily 3, group C, member 1], the mice do not get beyond gestation. One of the reasons we do not know very much about what the GCR does is because the mice never actually are born alive for us to do further testing on.

But what these nuclear receptors are responsible for is transcription of DNA genes to strands of mRNA, which are then translated (in the ribosomes) into proteins.

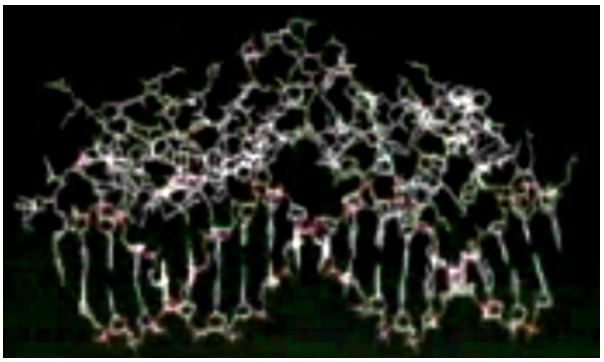
(Now, if you want some basic genomics tutorials, there is a simplified set of flash animations at this particular URL which I have found to be particularly simple to understand, and yet quite accurate.) (http://www.johnkyrk.com/)

So now we will look at some simplified 3D animations of these transcription molecules, just enough to give an overview of what the nuclear receptors do, and how the Corticosteroids, ARBs and Statins affect gene transcription. Corticosteroids now, because as we went further and further into this study, we widened out the scope of interest as to what we were looking for.



[00:24:48]
GCR Homidimer "zinc fingers"

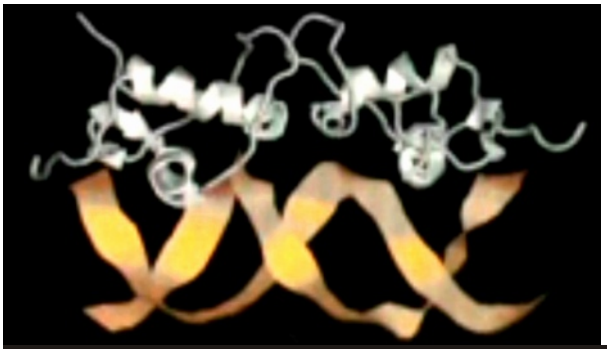
What I have there is a close-up of the skeleton of the GCR Homodimer. What that means is there are two GCR 'zinc finger' regions here, two proteins, and they are coupled together, actually, through this 'zinc finger' complex, and they are sitting on top of DNA. You can see the double strand of DNA here, and these helices — well actually underneath the zinc fingers it is hard to see; but that helix, and this helix, are responsible for causing the DNA bonds in the center of the DNA (which are all hydrogen bonds; there are not any molecules here in the center of the DNA strand) to break apart, by forces from the molecules here, and that causes the particular gene to be transcribed.



A very simplified explanation but it will do.

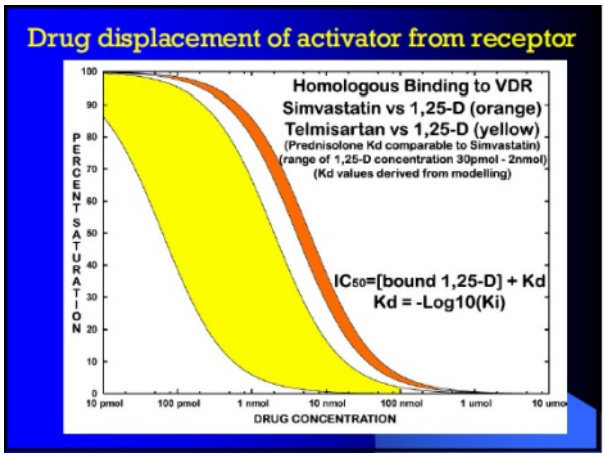
[00:26:07]
[3-D animation illustrations]

Now this is that same complex, but with a different perspective. You can see the DNA at the bottom. In this case, every single atom in the complex has been labeled.



We can select a different type of display here—we will just select the normal ribbon configuration. You can see DNA strands, and on here, or bound into here, is the receptor we saw on the previous slide. I just wanted to show you the back side of the DNA. You can see particularly the gaps across the center of the nucleic acids. Those are hydrogen bonds that fill those gaps. Those are what are broken by the nuclear receptors as they cause the gene transcription into RNA.

I do not know whether that is clear, but that was the clearest way I could think of to show what these things do. It is not that simple a concept. They are absolutely key to the operation of the cell.



[00:27:49]
Drug displacement of activator from receptor

Well, here is a graph that I am sure most of you will be a bit more familiar with.

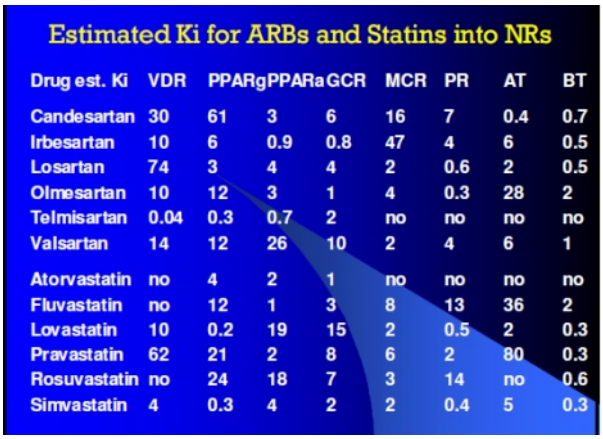
What we have here is per cent saturation on the left hand axis, zero to 100 per cent, and drug concentration across the bottom. We have the normal curve which indicates the IC50, which is equal to the bound natural ligand + Kd (where Kd is the disassociation function, or -log10(Ki). Ki, that is, for what we had on the other slide.

What I am looking at is the homologous bindings of VDR. In other words, we are not assuming any saturation of either the drug or the receptor. They are just homologous bindings to the VDR, with Simvastatin versus 1,25-D (which is in orange), and Telmasartin versus 1,25-D (which is in yellow). And I put a note there that the Prednisolone Kd is very similar to Simvastatin and it will have a similar displacement of the active 1,25-D from the VDR.

The reason the yellow band is so wide is because the concentration at the lower end of the yellow band is the concentration of 1,25-D in blood, and the concentration at the upper end is the predicted concentration inside the cell. It is quite a bit higher inside the cells of course, somewhere in the region of one to two nanomolar. That has been determined in vitro, approximately. The Kd value was derived from modeling.

You can see that as the drug concentration increases, the displacement of the original ligand, the active 1,25-D in this case, drops to zero—which is what you would expect. The more drugs you take, the more it is going to displace the active ligand; and disable, because these are all antagonists, disable the receptor.

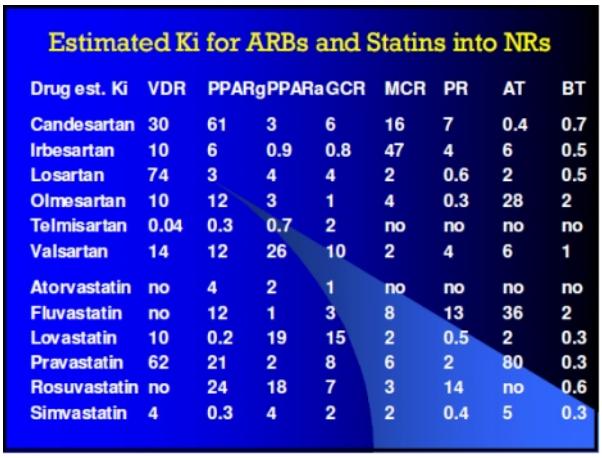
By the time you get to ten nanomolar concentration (one to ten nanomolar is typically what is used when these drugs are dosed in pharmacologic applications) that has significantly impaired the functioning of the VDR.



[00:29:59]
Estimated Ki for ARBs and Statins into NRs

So here is a table. I was told that the FDA loves tables—so here is a table. On the left we have got the various drugs; we have got the ARBs and then the Statins. Over the top we have got VDR, PPAR-gamma, PPAR-alpha, the glucocorticoid receptor (GCR), mineralcorticoid receptor (MCR), the progesterone receptor(PR), and alpha-thyroid (AT) and beta-thyroid (BT).

I have left off the estrogen receptors and the antigen receptors because, honestly, they do not change the overall picture very much.



Estimated Ki for ARBs and Statins into NRs

Drug est. Ki	VDR	PPARg	PPARa	GCR	MCR	PR	AT	BT
Candesartan	30	61	3	6	16	7	0.4	0.7
Irbesartan	10	6	0.9	0.8	47	4	6	0.5
Losartan	74	3	4	4	2	0.6	2	0.5
Olmesartan	10	12	3	1	4	0.3	28	2
Telmisartan	0.04	0.3	0.7	2	no	no	no	no
Valsartan	14	12	26	10	2	4	6	1
Atorvastatin	no	4	2	1	no	no	no	no
Fluvastatin	no	12	1	3	8	13	36	2
Lovastatin	10	0.2	19	15	2	0.5	2	0.3
Pravastatin	62	21	2	8	6	2	80	0.3
Rosuvastatin	no	24	18	7	3	14	no	0.6
Simvastatin	4	0.3	4	2	2	0.4	5	0.3

What you see looking at this table is that there are an awful lot of numbers which are below "1". Any of these numbers that are below "1" indicates that that drug is going to have a very significant impact on that receptor at the normal concentrations that these drugs are administered in pharmacology.

Some of them, for instance, Telmisartan, does not really affect the thyroid receptors, the MCR or progesterone receptors very much, but it really knocks out the VDR and PPAR-gamma and PPAR-alpha. The same with Atorvastatin. It has a strong affinity for PPAR-gamma and alpha, but not too much on the thyroids.

The thing that is really clear from this is that every ARB and every statin is a little bit different in its activity profile. So even though your clinical medicine looks at "a Statin" as being "a Statin," and really does not pay very much attention to whether it is Primastatin or Atorvastatin, Lipitor or so forth, there actually is a huge difference in their profile in terms of what receptors they are affecting in the human body.

But one thing that is common to them all is that all of the statins affect PPAR-alpha and PPAR-gamma. Some of the statins also affect VDR, notably Simvastatin, and Lovastatin marginally.

> "Any of these numbers that are below '1' indicates that ... drug is going to have a very significant impact on that receptor at the normal concentrations that these drugs are administered in pharmacology.

That is not unreasonable if you think about it. You have got a drug that is targeted at lipids, and it goes after the PPAR receptors. That could be the primary mode of action—we do not know. But certainly the job of an expert in genomics is to point it out, so that the in vitro work and the clinical work can go away and say, "Oh yes, that is true, this is a major function of this class of drugs, and the major reason why they act the way they do."

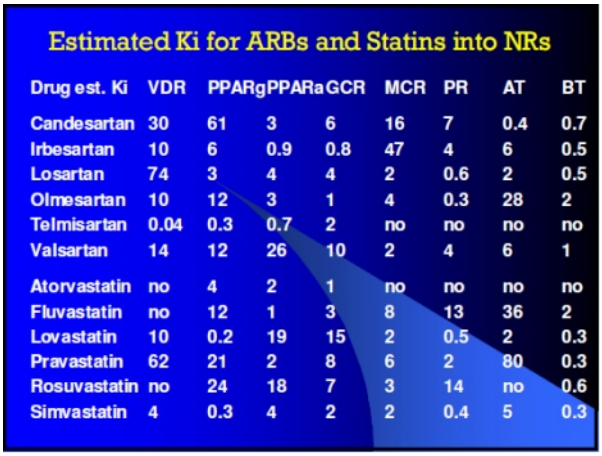
The same with the ARBs. Some of them have a high affinity for the VDR and PPARs. Candesartin, you see, does not have high affinity for VDR and PPARs, but have a look over here at the thyroid receptors. The thyroid receptor, Candesartin has a very high affinity to. At the other end of the screen you have something like Valsartan.

Audience Question: To what extent do we know about thyroid function? I see the numbers are all over the place. How much is thyroid function affected when these numbers are low, as we know that there are replacements?

Answer: With numbers that are around 0.5 and 0.7, that receptor is going to be almost totally blocked by the ligand. Now what effect that is on the body is a much bigger, imponderable question.

Audience Comment: That is what we are interested in!

Answer: I mean, you could look at it from two points of view. The first point of view is that really, these should not be doing anything in the nucleus. They should not be affecting the thyroid, for example. So that is the first observation you can make. And then the other observation you can make is, well, the people are sick. They need a drug. So—where is the middle ground? How can we select a drug that …



Estimated Ki for ARBs and Statins into NRs

Drug est. Ki	VDR	PPARg	PPARa	GCR	MCR	PR	AT	BT
Candesartan	30	61	3	6	16	7	0.4	0.7
Irbesartan	10	6	0.9	0.8	47	4	6	0.5
Losartan	74	3	4	4	2	0.6	2	0.5
Olmesartan	10	12	3	1	4	0.3	28	2
Telmisartan	0.04	0.3	0.7	2	no	no	no	no
Valsartan	14	12	26	10	2	4	6	1
Atorvastatin	no	4	2	1	no	no	no	no
Fluvastatin	no	12	1	3	8	13	36	2
Lovastatin	10	0.2	19	15	2	0.5	2	0.3
Pravastatin	62	21	2	8	6	2	80	0.3
Rosuvastatin	no	24	18	7	3	14	no	0.6
Simvastatin	4	0.3	4	2	2	0.4	5	0.3

Audience Question: Well now, we know the thyroid is being affected, there are medical steps that can be taken so that a person who knows giving this drug will have this effect, would be taking amelioration steps. Can they be sure they are not supplementing someone who does not need supplementing?

Answer: Absolutely. You can supplement thyroxine, for example. The major thing that is in my mind is that we need to be measuring and looking, so when somebody is being given Candesartan, for example, we should be measuring the T3, T4, and TSH. And if it needs supplementation with T4, with thyroxine, then that might be a good trade-off. That has to be judged by the clinicians, as to what trade-offs are there.

Audience Question: Was this assay done in membranes or cell culture?

Answer: This is mathematics—this is done in the computer. No in vitro work at all. (Okay?)

So what needs to happen now is, these numbers (which are very hard to determine in vitro or in a clinical environment) have to be kept in the back of our minds as we go back to the in vitro work and the clinical work, and figure out what to measure, what to look for, in order to elucidate the the number of functions of the drug.

"Olmesartan directly affects the VDR... clinical data... indicates that the levels of 1,25-D and PTH, and secondarily, the thyroid hormones, react to administration of Olmesartan. And yet in the NDA, Olmesartan is listed as not having permeability through erythrocyte membranes.

Audience Question: What clearly comes out is some of these compounds here are water soluble, they would not have the binding into the erythrocyte membrane, neither are they transported into the cell. One of the examples it comes out in is the Statins. You can see that unless it is delivered into the the phagocytes, it does not go into the cell at all, but I can see numbers very effective in nanomolar range. It just makes it hard to understand.

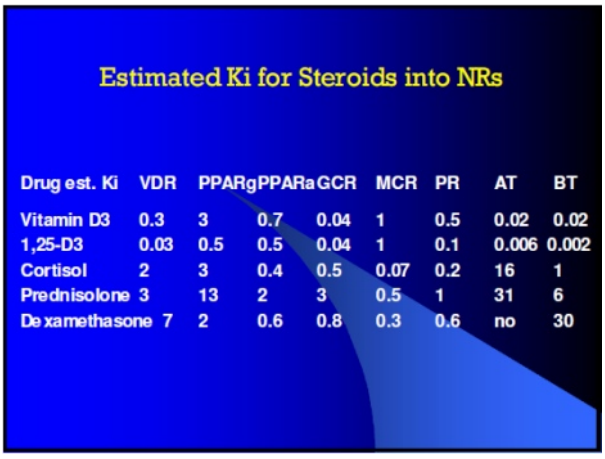
Answer: Well, I can tell you for certain that Olmesartan directly affects the VDR, because I have seen clinical data which indicates that the levels of 1,25-D and PTH, and secondarily, the thyroid hormones, react to administration of Olmesartan. And yet in the NDA, Olmesartan is listed as not having any permeability through erythrocyte membranes. So frankly, I think that is wrong. So, I think that what we need to do now is go back, and figure out how much is getting in, if any is getting in.

Let me put it another way. You are telling me that Olmesartan can not get into the phagocyte. And yet HIV can? You understand what I am telling you? It just does not stand the taste-test. It just does not sound right. And in the case of Olmesartan, we know it does get in because we have got some anecdotal clinical data.

That is why we looked at VDR—because it was reported that when people started to take Olmesartan, their 1,25-D levels plummeted. In one patient, it dropped in half within two weeks. So that is why we looked at VDR. I mean, you can look at other drugs with these receptors and they do not bind. But the ones that I am showing you on these slides generally have an affinity and that is why they

are on the slides. The ninety-nine point nine per cent of the computer runs that we did which did not come out with an affinity are not on the slide. Does that answer your question?

But yes, that is a very good question.



[00:38:20]
Estimated Ki for Steroids into Nuclear Receptors

But on the next slide, there really is a lot less doubt. These are all nuclear. These are all drugs which are active in the nucleus. The steroids are active in the nucleus. And so we know they are there. We know all of these are active in the nucleus. And we can see some very interesting things that the mathematics is telling us, in the genomics. We can see, for example, that 1,25-D3 (which is the active ligand for VDR) has, actually, ten times higher affinity for the beta-thyroid, and similarly for the alpha-thyroid. That lines up with anecdotal indications we have, that in fact, high levels of 1,25-D in sick people does affect their thyroid function.

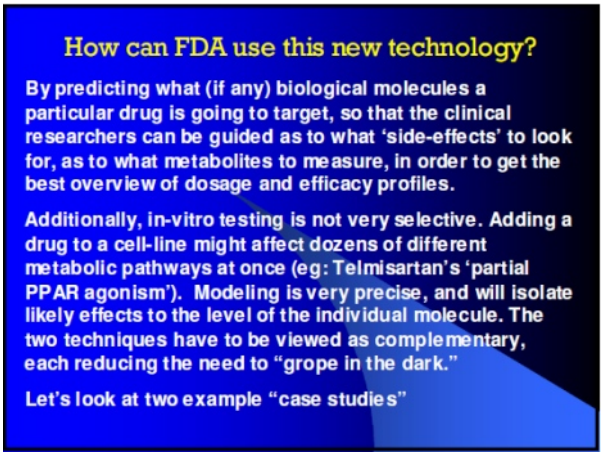
We can also see that cortisol, for example, has a different profile from prednisolone. For example, in the PPAR-gamma, there is a significant difference in affinity, whereas into the VDR they are about the same.

> "If you have got some information, use it. And use it to try and understand the bigger picture.

In the PPAR-alpha there is a significant difference, and there is not much into the alpha-thyroid, two to one. The amount of error that is involved in the molecular genomic calculations is quite high. If you get anywhere within three-to-one or so, and you have to regard the numbers as being essentially the same.

Dexamethasone also has a different profile from Prednisolone, which is interesting, and that could be clinically useful if it is kept in mind as clinical trials are performed. But you can see that dexamethasone to cortosol is somewhat more accurate, except in the thyroid receptors. It is somewhat more following the profile than prednisolone did.

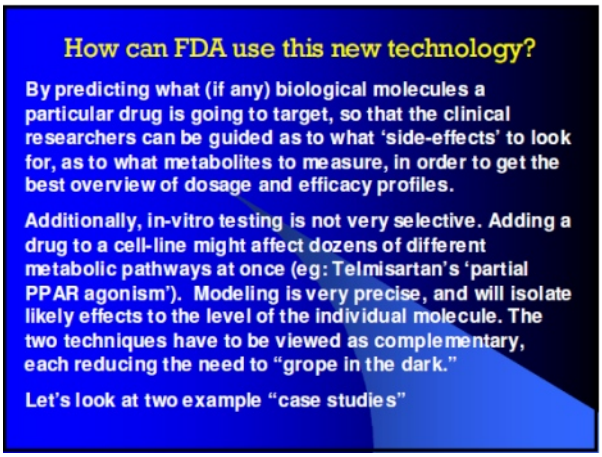
Then for completeness, I have shown the secosteroid vitamin D3 at the top. It is also active in all of these receptors, and as you can see, it also hits the thyroids pretty hard.



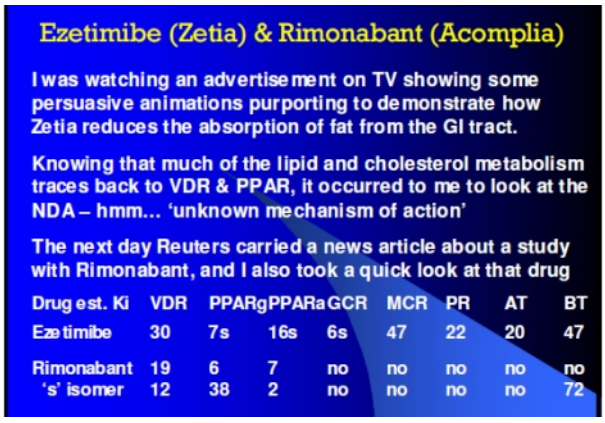
[00:40:30]
How can the FDA use this new technology?

Well, this is the question you were asking. What does this mean to the FDA?

Well, just as John Arbuthnot said: If you have got some information, use it. And use it to try and understand the bigger picture. There has been so much effort put into clinical trials of ARBs versus ACE inhibitors. But if the molecular genomics had been applied at an earlier stage to give insight into what to measure in these trials, what sort of end points could we expect? Then a lot of inefficiency would have been avoided, and the answers would be coming up a lot more quickly, as well.



"By predicting the biological molecules which a particular drug is going to target... the clinical researchers can be guided as to what 'side-effects' to look for... or to what metabolites to measure, in order to get the best overview of dosage and efficacy profiles.



By predicting the biological molecules which a particular drug is going to target, obviously the clinical researchers can be guided as to what 'side-effects' to look for, if they are unwanted, or to what metabolites to measure, in order to get the best overview of dosage and efficacy profiles.

For example, I have said there is one publication a day on the VDR. Yet I would doubt that there are very many applications before the FDA at the moment that even measure the metabolites that are affected by the VDR. And yet it is the key immune system receptor. It does key functions; and certainly it is key to any anti-infective capability that the phagocyte has. We need to watch these things. If a drug hits VDR, PPAR-gamma and PPAR-alpha, the clinical trials need to be told: "Be aware of infection; log infection."

Additionally, in vitro testing is not very selective. You can add a drug to a cell-line that might affect dozens of different metabolic pathways at once. And the art of the expert, the perfectionist, with in vitro, of course, is to stop this and try to focus in on the one metabolite that you are interested in. But it does not always work. In our paper we looked at a study which has shown that Telmisartan was a partial agonist of PPAR, and we pointed out that we felt it was far more likely that Telmisartan was affecting VDR, and since VDR was not being monitored in this particular in vitro experiment, that was why they were seeing the specific results that they got.

Modeling is very precise, and will isolate likely effects to the level of the individual molecule. But the two techniques are complimentary, each reducing the need to "grope about in the dark" as Arbuthnot said. They have to be viewed as complementary; and I think the example you gave: "Do these drugs even get into the nucleus?" is key to that.

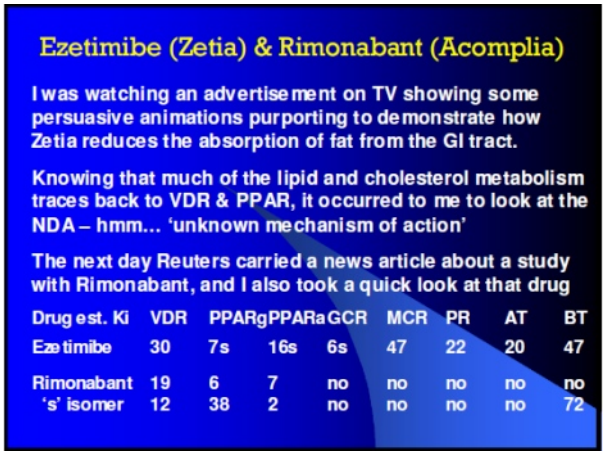
I mean, it has to be viewed from both points of view. If you assume that the drug does not get into the cell, either through the outer membrane or through the nuclear membrane, then the clinical trials will be looking for different sets of metabolites. But if you think there is a possibility that it might get in, you can measure the effects that it would have. And if you see those effects, you know that it is, in fact, getting in there and modulating the metabolism.

[00:44:24]

Ezetimibe (Zetia) and Rimonobant (Acomplia)

Now let us look at two example case studies. These were just a couple of drugs that came up while I was preparing this presentation about a week and a half ago.

I was watching an advertisement on TV which showed some persuasive animations purporting to demonstrate how the drug Ezetimibe (Zetia) reduces the absorption of fat from the GI tract. You have probably all seen these television advertisements. Since I know that much of the lipid and cholesterol metabolism traces back to the VDR and PPAR, it occurred to me to look at the NDA;

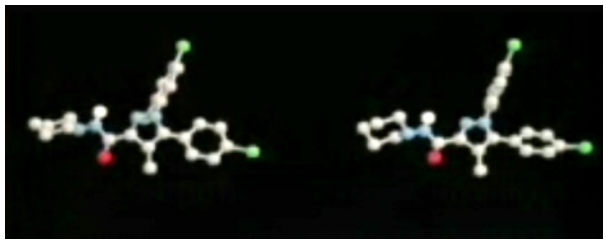


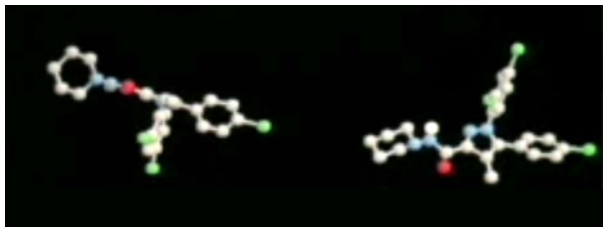
and when I looked up the NDA on Ezetimibe, I found "Unknown mechanism of action." So we ran a scan on Ezetimibe.

The next day, Reuters carried a news article about a study with Rimonabant. The study, if I recall, said that Rimonabant caused an average of fifteen pounds of weight loss within a year in a particular cohort. So I took a quick look at that drug as well.

Now, Rimonabant is actually fascinating, because if you were designing a drug to target VDR and PPARs, this would be the drug. You can see it has no effect on the other nuclear receptors (the 72 is negligible at the dosages used). One of the isomers has a very significant effect on PPAR-alpha, and the other isomer has a very significant effect on PPAR-gamma. And the effect of this isomer on VDR is moderate. It probably wouldn't show up at the 25 milligrams used in the trial.

Similarly for Ezetimibe. Just like the Statins, it affects the PPARs and affects them fairly strongly. 7 nanomolar is a fairly strong affinity at a typical dosage for Ezetimibe, which is also in the 25 milligrams a day region.





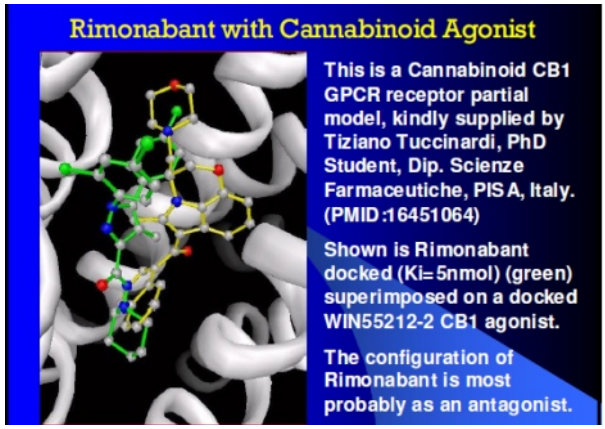
[00:46:44]

Isomers

Isomers. Those of you who know what an isomer is please bear with this. I just wanted to show everyone a quick slide to show what isomers are.

Isomers are when you have two configurations of a molecule. This is the molecule for Rimonavan. If I rotate the thing around … I have now rotated this backbone here so that the oxygen is facing towards us. You see that in one case, the benzene ring with the two chlorines is on the bottom, and in this case the benzene ring with two chlorines is on the top.

If I rotate it, you can see that where it is depends on the angle with which it is bound to this nitrogen. There is a hydrogen on this nitrogen (not shown, because you normally drop hydrogens out of molecules at the molecular genomic level. The computer knows where the hydrogens are.) But there is a hydrogen on this nitrogen and if it changes in location, then you get an isomer. It is the same drug, but it is a different shape.



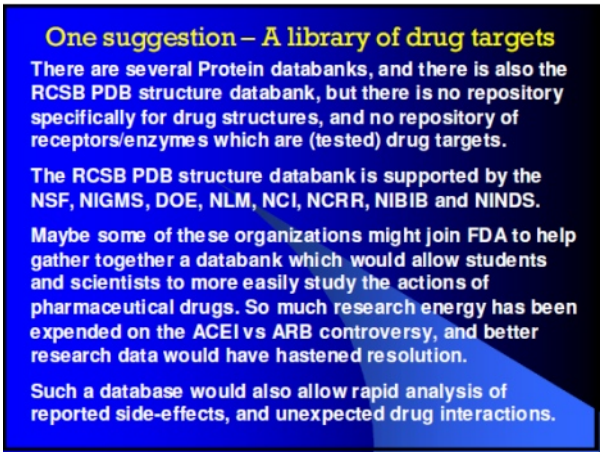
There are many enzymes in the human body that can change drugs from one isomer to the other. The classic case, of course, is Thalidomide. I am sure you have all been through Thalidomide, where one of the isomers, the one that we did not think existed in the human body, turned out to be teratogenic.

[00:48:02]

Rimonobant with Cannabinoid Agonist

Now Rimonabant does in fact go into cannabinoid receptors. It is a cannabinoid antagonist. I have shown that alongside the agonist, called WIN55212-2, which is a cannabinoid receptor type 1 agonist. You can see that they are not lying in the same general

location. They act on different residues. And Rimonabant is an antagonist, exactly as is stated in the press release from Reuters. The interesting thing is, it has about the same affinity for this receptor as it has for the PPARs.



[00:48:58]
Suggestion: A library of drug targets

So, I wanted to finish here by making a suggestion as to where we can go from here.

On the last slide, this cannabinoid receptor was supplied to me by Tiziano Tuccinardi, who is a PhD student at Pharmaceutical Sciences in Pisa, Italy. He had published a paper in PubMedIDs (PMID:16451064) on the cannabinoid receptor. They had produced a putative model and tested it fairly extensively, so I wrote to him and said, "Can you send me one of these receptors because I have got this drug that I want to try on the cannabinoid receptor and see what it does." So he sent it to me. I tried it and it docked in there, exactly as advertised.

But we should not have to do this. We should not have to track down these receptors. These receptors are key to the operation of the body. They are key to the operation of drugs. Why can't we have a database with at least the receptors, which are part of the human body, there is no copyright on them, no patent rights on them (well, mostly there is no patent rights on them.) Why can't we have a library of these receptors, just like the RCSB structure databank, so that people like the student in Italy that I got that cannabinoid receptor from, can deposit the receptor, and other students elsewhere in the world can take these and do what we have done with our study—show that, in fact, there is a significant spectrum of activity beyond what current medical knowledge portends?

"There is a significant body of information that we could be using to study the action of drugs in the human body.

There is a significant body of information that we could be using to study the action of drugs in the human body.

The RCSB databank is supported by science foundation and a whole stack of NIH and other bodies, including DOE. I was going to suggest that maybe some of these organizations might join the FDA to help gather together a databank which would allow students and scientists to more easily study the actions of pharmaceutical drugs. So much research energy has been expended on the ACEI versus ARB controversy, and better research data would have hastened resolution.

Such a database would also allow rapid analysis of reported side-effects and unexpected drug interactions, so that when a side-effect is reported that appears way off the wall, we can go back to the molecular genomics and say, "Well, is it that unreasonable, after all?"

In fact, I think today there is discussion of an MS drug which has an unwanted side-effect of allowing infection. If those drugs were screened against a known set of receptors and enzymes which are

known to be involved in the immune system, it would give us a very good starting point to work from for the clinical trials.

[00:52:24]
Question time

So I see that it is four o'clock, and I will be staying after the presentation to chat, and I would love to speak with any of you that are interested in this topic. I am also going to be here tomorrow morning (Wednesday the 8th) and I would love to talk with groups or individuals at that time. You might be interested in looking at the issues in more depth, or maybe just looking at the issues in the same depth, but looking at them again. So thank you very much for coming, and, I guess, that is it.

"We knew ... one of the ARBs seems to target the VDR. And because the VDR is key to the immune system ... we started looking ... into its actions.

Audience Question: How did you choose what the endpoint was for the immune system? Why did you say that the ARBs and Statins modeling do something?

Answer: Why did I choose the immune system? Well, we knew that at least one of the ARBs seems to target the VDR. And because the VDR is key to the immune system, at that point we started looking a bit more widely into its actions. That is really why we were talking about the immune system.

The immune system is, in any case, very closely intertwined with the lipid metabolism, the cholesterol metabolism in any case. It is very hard to separate them out. But I guess why I was surprised, was that these drugs that one would not expect to have any effect on the immune system at all, did—at least, on the computing level—have a very significant effect on key immune system receptors.

Audience Question: Have you modeled the biologics?

Answer: No.

Audience Question: Are you planning to?

Answer: The biologics are very large molecules and have an extra degree of complexity to try and model those. Yes, I have started to think about, particularly, TNF-alpha.

Audience Comment: I think it would give you some precision, I think, to begin to develop your thesis here. Because, for the most part, they are very targeted therapies as you well know. And determine which of these drugs could be dirty, could help you figure out where the modeling could fit in terms of the cart verses the horse, maybe.

Answer: Well, I think that it is important to note that these drugs *are* dirty, for a start. Because, I think, the average clinician out in the field has no concept that the drugs are dirty. So it is actually important to know the drugs are dirty.

Audience Comment: Actually, a lot of drugs are.

Answer: Well, yes, that is a whole job of its own just to get the word out.

Audience Comment: I would encourage you to do that, because to me, that seems to be the next level of what needs to go on here.

Answer: It is. You are dealing with much bigger molecules.

Audience Comment: Understood.

Answer: There are some issues with the way the modeling is done. Typically, we work on the receptor as being a fixed receptor. But no protein is fixed. It varies, the position varies from front to side, not only Van der Wiel forces but also the hemodynamic forces and other things. When you are dealing with the larger proteins, the modeling of the rings and the deformation of the rings becomes very significant, as it does with the steroids. The steroids are devilishly difficult to deal with because you have got the four ring conformation that you have to deal with.

"We just mastered the steroids and we thought that was great.

Audience Comment: But then, looking at the next iteration, we would be looking the various genetic snips, whatever, of the various pathways in TNF metabolism and such. Because that is where we are trying to figure this out. I am a Rheumatolgist. We are trying to figure this out with TNF inhibitors—and all the biologics that have been released. We think they are going to do something and then we just can not seem to find it, for example.

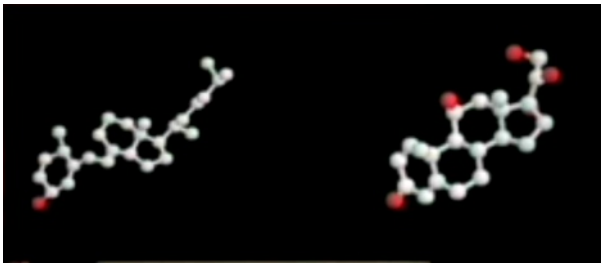
Answer: I have got this feeling—it is not a hypothesis, just a feeling—but there is something somewhere that activates all these GCPRs in extreme infection. Everything goes wrong; the eyes, the everything. Do not know what it is. TNF-alpha is always a good place to start looking, also Interferon-gamma, some of those. And yes, we do have that on the books, as it were, but it is an extra level of complexity beyond where we are at now. We just mastered the steroids and we thought that was great.

Audience Comment: Great. Thank you very much.

Audience Question: Is there a simple reference that goes through superficial layer the algorithm that was used goes in your computer estimation?

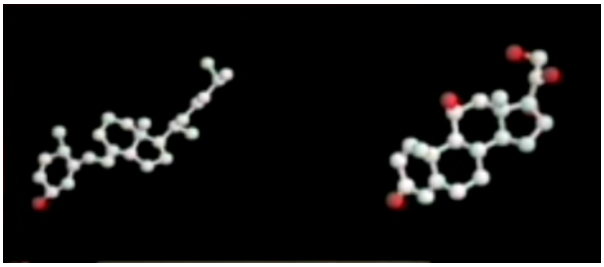
Answer: In our paper, well, the actual Ki, yes, there was paper published by a group at scripts that wrote that software, that goes into the Ki. I do not have it in my hand but I can certainly email you the reference.

Audience Comment: .... One does not think of it that way.



Answer: I know. It is because of history. This is what I wanted to show you. Here, This is vitamin D [left figure]. This is the steroid prednisolone [right figure],... OK, there are the two steroid rings of prednisolone and there is the methane on top of it. And here are the other two steroid rings. These rings are all bound together.

Now one of the things about the steroids that makes them effective is this is a very rigid structure, so it only fits in a few places. It does not fit in the angiotensin receptor, no matter how hard you try.



Now if you take vitamin D, this is pure vitamin D, not 1,25D, you can see the same two-ring structure here, and the same structure at the bottom, here, including the methane on the pole, but the difference is there is no bond across here [mid lower left of lower left figure]. These two atoms are not bonded. That is the only difference between them (25D and prednisolone).

Now, from the point of view of molecular affinity, that increases the affinity of this [vitamin D] molecule immensely, because these are all rotatable bonds, so it can twist and turn and get itself into those receptors very, very easily.

Audience Comment: I really like the examples you gave because it did make things clear.

References

1) Marshall TG: Molecular genomics offers new insight into the exact mechanism of action of common drugs—ARBs, Statins, and Corticosteroids. FDA CDER Visiting Professor presentation, FDA Biosciences Library, Accession QH447.M27 2006

Last modified: 08.11.2010