ANTHONY J. WINDEBANK: Good afternoon, and welcome to CTSA or CCaTS, as it now is, Grand Rounds, CCaTS, do-CaTS, whatever it is.

So it's a great pleasure today to welcome a longtime colleague and friend, Dr. Michael Fautsch. Dr. Fautsch is one of our few homegrown products here. He's originally from Minnesota. He attended college at St. Johns in Minnesota and then actually came to Mayo Graduate School, where he earned his PhD in biochemistry and molecular biology working with Dr. Eric Wieben here.

Now, often, when I'm introducing, I'll talk about how people have been attracted from far away. Dr. Fautsch is one of the people who went off to San Diego for a while and then actually came back to Minnesota. So we can applaud him for that and we're very lucky to have him here. He's a consultant and associate professor of ophthalmology and he's really internationally known for his work on glaucoma research.

Glaucoma's one of the world's leading causes of blindness. And Dr. Fautsch is also, I think, the model of the translational scientist who is taking and studying human tissue from people with disease and studying it at quite a basic level in the laboratory in order to come forward with new treatments.

And if he was coming into graduate school now, I have a feeling he'd come into the translational science track, but he did incredibly well in biochemistry and molecular biology. And we're delighted, Mike, to welcome you here. Thanks very much.

[APPLAUSE]

MICHAEL P. FAUTSCH: Well, thank you everybody for showing up this afternoon. I appreciate it. There are my slides.

There we go. So I've got to go through and tell you my disclosures. Unfortunately, I have no disclosures. So maybe someday-- we're working towards that. A couple of learning objectives that I'd like to go over with all of you today is I'd like to review the relationship between elevated intraocular pressure in glaucoma, as well as describe a novel class of compounds to treat glaucoma.

Now, the goal today would be to have you leave with a better understanding of what glaucoma is, some of the challenges ahead for us as we treat glaucoma, as well as describe or learn something about our research that we're doing on this novel class of compounds. Oops-- can't show that yet.

So like anything, what I want to do is tell you a story. And the story is still being written. I'm going to show you at the end of our talk today some of the preliminary data that we have as we move forward here. But this story involves a little serendipity, some good foresight, a willingness to collaborate, and most of all, the opportunity to work with a really dedicated, hardworking staff.

What you won't hear about are many of the failed experiments, a lot of the tribulations that we've had to go through-- a lot of the heartaches that we've had to go through in this type of research. And we won't go through something like these chaps have went through through the baldness center.

So for those of you that can't read it, it says, "Please remember that this is free research and study that we offered, that there were no guarantees for results." And I bring up the baldness because the person that I get my haircut from tells me I am going bald. So in a few years, I probably am going to lose a lot of my hair.

So like any good story, the plot is only as good as its characters, and I definitely have a lot of characters that work in our lab. But it's a fantastic staff. We've got a lot of different levels of experience. We go anywhere from-- oops-- Penny Kirgis right here, who's been at Mayo now just about 47 years, all the way down to Santanu, who's been in the lab now for about two months.

So we've got a variety of great experience, but it's a multi-disciplinary group. We've got Brad and Sherry that do most of our histology. We've got [INAUDIBLE] and Kim that do a lot of molecular biology. And then we've got Cindy, who is the world's expert at culturing human eyes, which I'll explain that in a little bit.

So the outline for today is I'm going to spend about a third of the talk and describe in my introduction kind of a little bit about what is glaucoma, go over some of the eye anatomy, what glaucoma is, some of the aqueous dynamics involved, and in the last 2/3 of the talk, I'm going to talk about what do ATP-sensitive potassium channels and glaucoma have in common. And I'll go over some of the research that we've been doing both on a molecular level, as well as looking at it from an ex vivo and an in vivo standpoint.

So all of you that are sitting out there, to be able to see me or to see my slides, there has to be light. So how do we see? Well, that light is reflected off any image-- for instance, if you're looking at me-- and it's going to enter the eye through the window of the eye, which is called the cornea. The light then travels through a clear fluid, which is called aqueous humor. And we're going to talk a little bit more about aqueous humor and this-- oops-- and this chamber right up in here a little bit because this chamber has to deal a lot with setting or regulating the intraocular pressure.

But as light comes through the cornea, it then is going to go through the colored portion of your eye, which is the iris, and otherwise known as the pupil. That works as a diaphragm, where it opens and closes and regulates the light that goes through your eyes. So as the light goes through the cornea, through this clear fluid, through the pupil, it then goes into the lens, where it's going to focus on the back of the retina, which is back in this area.

The retina, once stimulated with light, it's going to send electrochemical signals through nerve. And these nerves get together and form a bundle in the back of the eye, which is called the optic nerve. When we talk about glaucoma, glaucoma is a neurodegenerative disease. It's a disease of the optic nerve.

The majority of the work and the experiments that are being done on glaucoma, though, are involved in the front part of the eye, which is up here. Now, the reason for that will become a little bit clearer here in a little bit. But as Dr. Windebank mentioned, glaucoma is one of the leading causes of blindness. It's actually the second leading cause of blindness, but it is the leading cause of irreversible blindness. And it affects about 60 to 70 million people worldwide.

In the United States, 2.2 million people have glaucoma, but it's believed about four million people in the United States have glaucoma. And that's partly because glaucoma is also called "the thief of sight." It's one of those diseases that you don't really know you have it until you start losing your vision.

The right side of my slide here just shows if you were to take an opthalmoscope and look in the back of the eye, this would be what the normal optic nerve looks like right here. If you do a cross-section or that, it shows the histology here at the bottom, which you have a nice looking optic nerve with a lot of the different nerve bundles going through it. In glaucoma, though, what happens is you lose a lot of these optic nerves. And you can see here you get this excavation or pitting, otherwise called "cupping," that occurs in glaucoma.

Now, there are a lot of different types of glaucoma. It's a heterogeneous disease. There are mainly two classes. One is a closed angle. The other one's an open angle.

This image on the right here shows, if you look at B, here's a closed angle, which is mainly an anatomical problem where there's actually a closing off of some of the structures where fluid is drained from the front part of the eye, where the open angle is not due to an anatomical problem. But it's actually due to a variety of different problems and that's what we're studying in the laboratory.

There are a lot of different forms of open-angled glaucoma. There are congenital forms and juvenile forms that mainly have a genetic component to it. And then there are adult forms, and the most common form is Primary Open-Angle Glaucoma, otherwise called POAG. Now, there are multiple risk factors for the disease. Age is a particular risk factor.

If you can see up here at the top, on the x-axis is age in years and the incidence of glaucoma. This is actually in Olmsted County. You can see as individuals get older, their incidence of glaucoma goes up. And actually, in the decade in the 70s, this is approximately one in 500 people have glaucoma.

There's also a family history-- diabetes. The most prevalent risk factor for glaucoma is elevated intraocular pressure. And here, you can see on the bottom, again, on the x-axis, we see age in years. On the y-axis is increasing in pressure. And you can see how as you get older, you increase elevated intraocular pressure. If you were to look at individuals that had glaucoma, you would see a different slope. It would be a much steeper slope. It would start somewhere up in here and it would be much more steep-- much higher pressures as you get older.

Now, if you do not treat glaucoma, you will go blind. And as I mentioned before, this is called "the thief of sight." And partly, that happens because as you can see here, this is a picture of actually my three daughters. This is what they normally look like. Actually, they act like that a lot.

But in early stages of glaucoma, again, you don't realize you have glaucoma because what you start doing is losing your peripheral vision. As most people start losing this-- again, it's very gradual. It happens over years-- you start to accommodate for that. You might start turning your head a little bit more to see. You don't really realize you have it because you're not going to have any pain.

But if you don't go in and get regular eye check-ups, you're not going to be able to know that you have elevated intraocular pressure. And even though family history is a very important risk factor, anybody can get glaucoma. We just don't understand enough about the disease to know what causes it. All the treatments, every treatment that's out there right now, is geared towards lowering elevated intraocular pressure. There is no cure for the disease.

Now, if we look at how intraocular pressure is produced or how you get intraocular pressure in the front part of the eye, there's a couple different structures that I just want to briefly mention because you're going to hear a little bit more about them. One is the ciliary body, which is where aqueous humor is actually produced. And then you also have what's called the trabecular meshwork. And this is mainly the tissue that we study in the laboratory.

The trabecular meshwork is a filter apparatus. So it actually filters aqueous as it moves from the anterior chamber through the trabecular meshwork. And then it drains into a small canal called Schlemm's Canal. This canal then hooks up through a variety of different collector channels where fluid drains through these collector channels into aqueous veins and then eventually into the episcleral venous system.

So this is what that looks like. Again, ciliary body produces aqueous humor. The fluid flows into the anterior chamber, then goes out mainly through the trabecular meshwork and Schlemm's Canal. Now, for IOP, to have an Intraocular Pressure, there's a balance between the amount of aqueous humor that's produced and the amount that's removed. When this balance gets interrupted, you get either a decrease in intraocular pressure or an elevation in intraocular pressure.

What happens under the glaucoma condition is it's pretty well established that the amount of aqueous humor does not change in people with glaucoma or normal people. Where you see the problem is in the removal. For whatever reason, it's very difficult to remove or it's more difficult for people to remove aqueous humor from the front part of the eye.

Now, there are treatments, as I mentioned, for this, but all of them are geared towards lowering intraocular pressure. There are a couple surgical methods. One is a laser method where they actually use a laser to open pores in the trabecular meshwork to increase fluid flow through the trabecular meshwork.

There's also a filtering surgical procedure where there actually is a hole drilled through the sclera and it actually takes a little small chunk of the trabecular meshwork out. And this allows fluid to drain from the front part of the eye actually to the outside of the eye.

There are also therapeutic treatments, and this is really the first line of treatments if you'd come in and have elevated intraocular pressure. There are some that work on the amount of fluid that's produced-- so on the inflow here in the ciliary body. But then there are also drugs that are used to increase outflow.

The nice thing about these drugs is that they lower intraocular pressure. The bad things about the drugs is that they don't protect the optic nerve. So there are no drugs out there-- and again, if you remember, this is a neurodegenerative disease. So this is a disease of the optic nerve. There are no drugs out there right now that help protect the optic nerve.

So the holy grail drug-- of course, a holy grail would be to cure the disease. But the holy grail drug would be to identify a drug that can lower interlocutor pressure, which is now treating the site or the main risk factor for glaucoma, as well as then protect the optic nerve from damage. So that would be the perfect drug.

So this is just another image, a histological section showing what the trabecular meshwork looks like. This is actually the anterior chamber. It shows that the majority of outflow happens through the trabecular meshwork. This is actually the trabecular meshwork right here. This is Schlemm's Canal.

And then there is another alternative pathway, which is called the uveoscleral pathway. In people, as you get older, as you can see, the majority of outflow goes through the trabecular meshwork. This increases through this pathway as you get older, hence again, with primary open-angle glaucoma, with an age, increase in primary open-angle glaucoma. That's part of the reason why we're seeing individuals that are older that have more problems trying to get fluid out of the front part of the eye.

Over on the right side is a scanning electron micrograph. Now, the trabecular meshwork, as you can see, it's actually a filtering apparatus. If you see down here in the bottom right, you can see that there are little pores. And these pores actually get smaller as you go through this trabecular meshwork.

It was about 50 years ago that Morton Grant did a study looking at glaucoma eyes. And I won't go into the study in detail, but what he actually found was that the site that's listed here in the red box is really the site where the problem is in removing fluid from the front part of the eye. So it's in between the trabecular meshwork and Schlemm's Canal. So this is where the majority of resistance is in primary open-angle glaucoma.

So what does my laboratory do? What are the interests? So my main question in the laboratory is, how is intraocular pressure controlled in the normal eye? We still really don't know the exact mechanism and how fluid moves from the anterior chamber into Schlemm's Canal. This was first identified back in the 1870s, but here we are 130 years later still trying to identify if it's a passive process or if it's an active process.

So there's still a lot of work to be done. And of course, the second question is, how is this changed in glaucoma? And we've got a variety of different projects going on in the lab. I'm not going to talk about these individual projects today. But again, we do a lot of molecular studies, as well as histological studies.

We also have a very interesting project going on looking at the effect of cerebrospinal fluid or intracranial pressure and its role in glaucoma. But I can talk about that, if anyone's interested in that, later on. What I want to talk about today, of course, is modulators of intraocular pressure. And this is where our results and our work has been done, looking at these ATP-sensitive potassium channels.

So over the past 10 years, if you would have looked at this list 10 years ago, there would have probably been about two different molecules on this list. So there have been a lot of molecules now that have been associated with altering intraocular pressure. Some of these, when their expression is increased, have roles on increasing intraocular pressure, such as TGF beta 2, which TGF beta 2 is actually the main transforming growth factor that's found in aqueous humor.

We also have connective tissue growth factor. Gremlin's another molecule that if you have increased expression of that or if you overexpress gremlin, you'll see an increase in intraocular pressure. But there are other molecules, too-- for instance, like the Hep II domain of fibronectin-- which also decrease intraocular pressure. We're adding to that list, which is the ATP-sensitive potassium channels.

So how did we get involved in these ATP-sensitive potassium channels? It's actually a very interesting story. And when I've visited other institutions, I've always wanted to tell this story because I think it's great for any students involved to always look at what your data tells you. And this particular story started back in 2008.

And we do a lot of research for pharmaceutical companies. We've got a model in the laboratory where we can test different drugs, different proteins, to see if they have an effect on intraocular pressure. So a lot of times, pharmaceutical companies will contact us and see if we can test their particular drug.

We got contacted in 2008 from a really small company out on the East Coast. And they had a compound that they were looking at that they were looking at it because it had neural protection properties. And they were looking at it in a rat model. But what they had noticed is that they thought that there were some changes in intraocular pressure.

So what they did, they sent us a compound. We tested it in our culture model and lo and behold, it had a fantastic effect. It lowered intraocular pressure. Wow, we were really excited about this. We were talking to the company. We were in the process of writing grants.

Well, if you take a step back and look at 2008, 2008 is when the Great Recession took place. And unfortunately, very few venture capitalists were putting money into new companies. And again, this was a very small company. Long story short, the company went under. We didn't have any access to the drug, the drug went away, and the only thing that we knew about the drug is that it was a member of the potassium channel family.

We knew very little else about it. So me being a personal molecular biologist, I have to admit, channel biology was not one of my fortes. Fortunately, my next door neighbor up on Guggenheim Nine, Dr. Mike Romero, has a laboratory right next door and he is a channel guru. So again, this is where the collaboration comes in. And that's where Mayo's such a great place because we have these interactions on different floors. And Mike and me have been working on this project now for the last few years.

But I actually went to the literature, started reading a lot about potassium channels, potassium channel families, and there are really four different channel families. There's the tandem pore domain, which is mainly involved in resting potential. We have the voltage gated potassium channels, which are involved in action potential. There are some channels that are regulated by calcium and then there's these inward-rectifier potassium channels.

And the class that really struck me was these potassium channels that are ATP-sensitive. And the reason they struck me is because these are actually cellular biosensors. They actually sense the cellular metabolic states of cells. And as cells are normally healthy or have an increased cellular metabolism, you're going to have a fair amount of ATP around. In that situation, these channels are closed and that's really the normal state of most cells in your body.

Under stress, under conditions that the cells aren't happy with or a decreased cellular metabolism, these channels will open up. These channels are also unique to the potassium channel family because there are two different subunits. Most potassium channels just have one subunit, which would be like the Kerr subunit.

In this situation, there are actually two different subunits. One is the Kerr family, which there are two different members, 6.1 and 6.2, as well as the SUR subunits, which there are actually three different ones, SUR1, SUR2A--SUR2B. SUR2A and SUR2B are from the same gene. The only difference is a 42-amino-acid difference between 2A and 2B. So you can see if you put these different channels together, you can come up with a variety of different channels within this class.

What is known is that if the Kerr channel is Kerr 6.1, all four subunits, which you can see here in my diagram-- these channels are actually octomers-- these four subunits will all be Kerr 6.1. For a SUR, then they'll either be all SUR1, all SUR2A, or all SUR2B. So what you can get is six different channels. You can get a Kerr 6.1 interacting with SUR1 or SUR2A or SUR2B and the same thing with 6.1.

So it's an opportunity to really-- there can be a lot of variety and a lot of different functional differences between these channels. The other thing that struck me about this class of compounds is the cellular functions. These cells or these channels in different cell types have been shown to have contractile properties of smooth muscles.

They're also involved in the formation of gap and tight junctions. They're involved in actin cytoskeleton rearrangement. They have cellular endurance and recovery during stress. All of these potential functions have in one way or another been associated with glaucoma or the movement of aqueous humor in the front part of the eye or the decrease in it.

There are also available drugs that open and close these particular channels. There are KATP channel closers, which are called glyburide and tolbutamide, which mainly are used clinically today for type 2 diabetes. But there are also KATP channel openers, which is what mainly that we're looking at.

And these are diazoxide, nicorandil, and there's a whole variety of these different compounds. And mainly, these openers are going to bind to different subunits within the channels. Diazoxide is mainly used for hypoglycemia, but it's also used in acute hypertension situations.

So what do ATP-sensitive potassium channels and glaucoma have in common? Well, the first question you have to ask is-- well, before that even-- is, what models do we use in glaucoma? And unfortunately, there are no animal models to study primary open-angle glaucoma. And the main reason for that is the main risk factor for primary open-angle glaucoma is increased intraocular pressure.

So the only way that you can really do that in an animal standpoint is you have to destroy the outflow pathways so you can't get fluid out. If you destroy the outflow pathway, now it's really hard to test that drug to see if it helps the outflow pathway. So there are no animal models and there are very, very few occurring models.

A few different subsets of beagles have elevated intraocular pressure and have glaucoma-like symptoms, but again, it's not a primary open-angle glaucoma. So we can't really use any animal models, so we have to look at molecular studies looking at monolayer cells. And what we do there is we actually can go into human eyes and actually strip out the trabecular meshwork.

Now, to let you know how much tissue that we're dealing with is think about taking a fish line or a fish string and putting that around the neutral cornea. That's what we're dealing with, a very, very small amount of tissue. There are about 200,000 to 250,000 cells in that tissue-- so very little tissue. But we can take that out and we can make primary cell lines out of that tissue.

There's also an ex vivo model that I'll talk about here in a little bit. And we do use animals as models, but again, we have to use normal tensive animals. We can't use anything that has elevated intraocular pressure because, again, we have to destroy the outflow pathway. So I've talked a little bit about the structure.

And the only thing I wanted to mention here again is just to reiterate that we can get different types of channels. And that's shown over here on the right side, where you can get combinations like of Kerr 6.1 and SUR2B, which are found mainly in epithelial non-vascular cells. Or you can get Kerr 6.2 and SUR2B, which are mainly found in non-vascular smooth muscle cells.

So the first thing that we had to do in the laboratory is ask, well, OK, if these channels are actually involved in regulating intraocular pressure, do any of the cells of the outflow pathway have any of these subunits? So we looked at these from a molecular standpoint, did PCR looking at all the different subunits, and found that for the most part, most of them are there. SUR2A, SUR2B, Kerr 6.1, and Kerr 6.2 transcripts were found by PCR. We did not find SUR1 and that is not of a huge surprise because that's mainly found in the pancreas, as well as some cells in the heart.

When we looked, now, it's great that it has a message, but does it actually have protein? And so we went on and did some immunohistochemistry. So the top part of this image right here shows tissue that we've actually isolated from human eyes. So this is the trabecular meshwork here, the anterior chamber would be down here, and Schlemm's Canal, which is the SC region, right up here.

This is actually of SUR1, which, not surprisingly, we don't see any protein. This is SUR2A. This was a surprise because we did see some transcript there, but we did not see any protein there. Now, this could be due to antibody issues, as well, because the difference between SUR2A and SUR2B is only 42 amino acids.

And we've looked at several different antibodies that are out there and we've even struggled to see in our positive tissue any signal. What we can say for sure is that SUR2B is there, as you can see in the green here, as well as Kerr 6.1 and 6.2. The bottom part is just showing you some immunohistochemistry from our trabecular meshwork or our primary cell lines.

We then went on to look at KATP channel activity in TM cells. It's great to have the subunits, but do the channels actually work? And this was actually worked on in Dr. Romero's lab by Dr. Chang, who's also sitting in the front here. And here, we did some patch clamp looking at normal trabecular meshwork cells. These are monolayer cells.

And so we're looking at channel activity. And what you can see here, under a control, we have very little channel activity at the different currents. And that's not a surprise because these channels are normally closed. When you add now DZ or diazoxide, one of those KATP channel openers, what you see now is you get a lot more activity, a lot of increased activity, suggesting that these channels are there and that they're functional.

We then went on to add the diazoxide, which opens the channels. But we also added in glyburide, and glyburide is a potassium channel or a KATP channel closer. And it's been shown in other cells that the closer is actually dominant over the opening function.

And not surprisingly, you can see that when you add the two together, again, you reduce the activity pretty substantially, which suggests from all these studies that diazoxide increases electrical conductance and glyburide inhibits that or the closing of those channels inhibits the diazoxide activity. So now we have subunits there and that they're functional.

So fortunately in our laboratory, we've got an ex vivo human culture model. This model was developed here at Mayo back in 1987 by Dr. Doug Johnson. And this model actually involves taking a human eye-- and we get our human eyes from the Minnesota Lions Eye Bank, but greater than 95% of them come from donor patients here at Mayo Clinic. So we're very fortunate to have that opportunity to get eyes here.

This particular model takes an eye and we can dissect it at the equator. So we just have the front part of the eye. We can put the back part of the eye-- we can fix the back part of the eye so we can look at it later. But we take that front part of the eye. We take out the lens, the iris, and the vitreous, and then we clamp it in a modified Petri dish.

And this is depicted here with a modified Petri dish. We can then clamp that down and then we can perfuse in media at the normal flow rate, which is 2.5 microliters per minute. Once this fills up and gets full, we now have a closed system. There's only one way for the fluid to go out of this system and that's through that trabecular meshwork-Schlemm's Canal pathway.

Through a second cannula, we actually have this hooked up to a pressure transducer. So we can actually measure the pressure in each one of our eyes. And this is just a little graph showing you. You'll see a few of these here in a second.

So this is a fantastic model. Dr. Johnson actually went up and set it up into about 10 different labs throughout the world, actually, in Germany, France, multiple places here in the United States, and it's the only human model that's available to look at the effects of intraocular pressure or to test what drugs or molecules can affect intraocular pressure.

This is, again, Cindy [INAUDIBLE], who works in my laboratory who really is the world's expert. She has cultured-- Cindy, you're going to have to help me here-- probably about 2,500 pairs of eyes now in the last 20 years or so. But this is actually our set-up, where we have a perfusion pump here. We've got our different syringes with media.

Again, these are perfusing media in at about 2.5 microliters per minute. And here are our eyes. And then actually, through a second cannula, here's our pressure transducer. And this is hooked up to a computer so we can actually measure the pressure in these eyes in real time.

So when we look at this-- and I'll ask the question, what happens when you add diazoxide? What happens when you add diazoxide? Does it have any effect on intraocular pressure? So here's a pair of eyes where we have pressure on our y-axis. And then on the x-axis, you're going to see this is time.

So this is a pair of eyes. It's from the same individual. We always work in pairs. What we did at this given time point here, once the eyes have been stabilized, their pressure stabilized, we added diazoxide into one eye. And then, of course, our vehicle control is in the other eye.

And as you can see, over time, we get a nice decrease in pressure in these particular eyes. And this is looked at over a little over 36 hours, about 48 hours. At this time, if this is a real effect, we should be able to take the drug away and pressure should be able to go back to baseline.

So that's exactly what we did. We took the drug away and you can see that pressure comes back to baseline. And so this is really an exciting result and we've moved this on. We've actually looked at eight pairs of eyes doing this. And as you can see, this is looking at pressure changes.

So in our treated eye, where our baseline was right around 17 millimeters of mercury, after adding the drug, we went down to 10 millimeters of mercury, where you can see in our baseline in our vehicle, we had relatively no change at all. Another way of looking at-- not looking at intraocular pressure, but is looking at outflow facility. And that's the ease in which fluid moves through the outflow pathway.

And this is just showing this. And so an increase in outflow facility means that more fluid's getting through so it's a lot easier getting through. And you can see here that when you look in the change in our outflow facility in the eyes where we added diazoxide, we got an increase in every one of the eyes that we looked at, which is shown here in this, I guess, peach color.

We also looked at a couple different KATP channel openers, nicorandil, P1075-- we've also looked at cromakalim, which I don't have on this graph. To make a long story short, the KATP channels that we looked at, they all were able to increase outflow facility or decrease intraocular pressure. This is the histological findings because, of course, that's really important.

What did the drug do to the tissue? So we always look at every one of our eyes because this model system's a fantastic model system, but we do get about 25% failure rate. That could be partly due to putting the eyes in culture, to depending on whatever the length of time, from death to the time that we get them, the postmortem effects, as well as it could also be disease-related a little bit based on what the individuals had.

But when we look at this, when we looked at the eye with diazoxide, here's Schlemm's Canal. Here, Schlemm's Canal's lined with cells, endothelial cells. Here's the trabecular meshwork. And if you compare that really to the control, for the most part, I can tell you there's very little change. We don't see any toxic effects.

When you looked at this from a transmission electron microscopy technique, we see the exact same thing. Actually, it's very interesting. When we do this in the laboratory, when we do these particular studies, we always bring them to a lab meeting. And for the most part, every one is masked.

We don't know which one was treated and which one is not when we look at these. The only person that does is the person that's been doing the work. Or actually, Cindy is the only one that knows which one was treated.

Generally, when an eye's not looking very healthy, you would think, hey, that's the way that's treated. In this situation, it was just the opposite. The eyes that always were treated always look better, always look better than our control. And part of that, I think, as we've went on and on is that diazoxide, that's one of the effects. It actually has a cell-protective effect on many cells.

And the vehicle that we have to use to add this drug is Dimethyl Sulfoxide and of course, cells don't like DMSO. So it's not surprising that our control would look a little unusual because of the dimethyl sulfoxide. But the diazoxide, to look so good and so healthy, suggested, of course, that diazoxide was able to inhibit that effect that DMSO had. So that was a great result, as well. So we're not seeing these toxic effects.

We then moved on. And again, if you remember the experiment that we did looking at the effect of patch clamps and the functionality of these channels, we used the closer called glyburide. We then took six different eyes and we treated one eye with diazoxide, just like we had shown before, where we see this decrease in pressure.

But then we treated the other eye with diazoxide and glyburide. And again, when you added glyburide, glyburide's kind of dominant over the opening of these channels. We didn't see any change in pressure. We then, again, removed the drugs, put it back to baseline, and then we did a reverse experiment. So the eye that received diazoxide and glyburide first, which is shown here in the red, we then added diazoxide.

And as you can see, it is responsive so it is functional to the drug, where the other eye, when we added diazoxide and glyburide, which originally showed a decrease, when we added the two together now, the glyburide inhibited that. So again, this is another great result, again, showing more specificity for these particular channels in this effect.

So what happens when you go to in vivo? Of course, and in vivo's where we really want to be. We did a 28-day experiment and we used C57BL6 mice. We pretreated these mice for seven days. And really what we did is measured intraocular pressure at three different times during the day. Intraocular pressure can vary as the day goes on, so it's very important to do multiple time points.

We then took those three measurements and did an average to give us our daily intraocular pressure. And we did this for seven days just to get a baseline for each left and right eye. We then for 14 days treated one of the eyes with drug. In this situation, what I'm going to show you is diazoxide. And then the other eye, we treated with vehicle.

And then we looked at the difference between the pressures between the left eye and the right eye. So these particular drugs we treated one time in the morning. We added a five-microliter bolus of drug that was on the eye for anywhere from three to five seconds and then was washed out. And then we measured intraocular pressure at one hour after treatment, four hours after treatment, and 23 hours after treatment.

And then, of course, we took the drug away and then did IOP measurements for seven days after that. So these are the results. So if you look at our pre-treatment, we had very little change between the left and the right eyes, which is what you would expect. But after adding the drug, you can see we have about a 20% to 22% change in pressure. And that's a decrease in pressure in the eye that was treated.

Now, to give you a little bit of idea, if you look at the drugs that are out there on the market, some of the prostaglandin analogs particularly that work on the outflow pathway or some of the drugs that work on the inflow pathway, if you look at their results in mice, you'll see anywhere between a 15% and 25% change. So these changes are very good, very comparable to what you're seeing with some of the other drugs.

Of course, then we took it away. Well, this is the histology showing that there really is no change. And of course, we took it away and the drug came back to baseline. What's interesting here is it took about 48 hours. Once we added the drug, it took about 48 hours for the animal to actually get down to a maximal IOP reduction and it took another 48 hours for the drug to kind of make its way through the aqueous humor and get the IOP back to baseline.

This is somewhat surprising because aqueous humor turns over about every two hours. So there's only about 250 microliters of aqueous humor in your eye. And so this is going to turn over about every two hours. So it's interesting that you add this drug and it has an effect for 48 hours.

The reason that we started using mice in these initial experiments is because we were fortunate enough here at Mayo that Dr. Andre Terzic has been working on ATP-sensitive potassium channels for many years. But he's been looking at them in the heart and it just so happened that he has Kerr 6.2 and Kerr 6.1 knockout mice. So we were able to use mice that he gave us that had knocked out one of these subunits in these channels.

So we did the exact same experiment. And so the red here just shows what I've been showing you in wild-type. And then if you look at what happens when you add diazoxide to these particular mice, you'll see that there's no effect. We have no effect on intraocular pressure-- again, showing a lot of specificity for these compounds for these particular channels and their involvement in intraocular pressure.

So we've more recently been looking at, so how does this effect happen? We know that we've got a functional result showing that intraocular pressure can be decreased by adding these drugs. So what is the cell signaling pathway? How are these drugs doing this? These channels are opening and then what happens?

So what we did is we took lysates from monolayer cells that were treated with diazoxide and actually probed them and used a phosphoprotein array. And this has 510 different molecules. It's an antibody array that has antibodies that are involved in a variety of different signaling pathways, phosphoproteins-- some are not phosphoproteins. And we just then did a comparison between what our control had, as well as what our treated had.

To make a long story short, what we found-- whoops, going the wrong way here. What we found is that there were a variety of molecules that were up-regulated after we added diazoxide to these cells. And many of these, focal adhesion kinase, PKC, RAF, MEC-12. ERK1/2, and even Jun and Fos transcription factors, almost all of these are involved in the ERK1/2 pathway.

So we went on to look at if ERK1/2 was actually phosphorylated after adding the drug. And as you can see from this result here, here's our control at 0 minutes. Again, these were in monolayer cells. Here's the control, which is just the vehicle by itself. And then this is after adding diazoxide and looking at phospho ERK.

You can see there's a nice stimulation of ERK after 15 minutes. We added then an inhibitor which inhibits the ERK1/2 pathway. But really what it does, it inhibits MEC kinase. And MEC kinase is the kinase that actually phosphorylates ERK1/2. So by adding an inhibitor to MEC-12, if this is actually working through the ERK1/2 pathway, you should not get a signal. And that's exactly what we see over here with U0126 plus diazoxide.

So again, this shows more evidence that this is actually working through the ERK pathway. We've actually looked at this in mice, where we've treated the mice and 15 minutes after treating the mice, we went in and isolated the anterior chamber and then looked at ERK phosphorylation. And again, you see a very similar result. You see an increase in ERK phosphorylation.

And we also did this in our culture model, our human eye culture model, where we added in diazoxide. And with the culture model, we did this a little later. This is actually 14 hours after adding it in. And again, you can still see that ERK1/2 is actually up. So we're getting some ideas in regards to the molecular pathway that's actually involved with this channel opening.

We then took and asked the question, well, if that really works-- OK, if we're getting this ERK phosphorylation, so what's going to happen with intraocular pressure? If the KATP channel openers are actually working to decrease pressure in our culture model, we should be able to add in U0126, which will inhibit ERK1/2 and not get a decrease in intraocular pressure. And that's exactly what this is showing right here.

You can see here's our stable baseline here. Again, we have pressure on the y-axis, time on the x-axis. In this particular eye which is showing in the red, when we add diazoxide, we get that decrease in intraocular pressure. When we now add diazoxide plus the inhibitor of ERK1/2 pathway, you can see now that pressure goes back to baseline. Again, now we have functional data that suggests that this is working through the ERK1/2 pathway.

We did this, again, in multiple different eyes and this is just showing the change in outflow facility. Again, outflow facility is the ease of fluid getting through the front of the eye and so an increase in outflow facility is a good thing. That means there's a decrease in intraocular pressure. Our baseline is very similar.

In treated, of course, we're going to get that nice increase in outflow facility. When you add U0126, again, it's nearly back up to baseline-- so again, more evidence suggesting this is working through the ERK1/2 pathway. So now that we've got molecular data suggesting the pathway, from a physiologic standpoint, the big question was, how is this actually working? When you add in diazoxide, what's actually happened?

And so there's a couple of different theories on how intraocular pressure is actually controlled. One of them involves looking at the ciliary muscle contraction and the TM relaxation. And this is depicted here.

So here, the area here on the left is a depiction of the trabecular meshwork. An area over here is the ciliary muscle. It turns out that the ciliary muscle has tendons that run through the trabecular meshwork and actually up to Schlemm's Canal. And as the ciliary muscle contracts, the TM actually relaxes. And once the trabecular meshwork relaxes, more fluid can actually get through the trabecular meshwork, which is shown over here on the right side.

So if this is true and if this is the pathway that KATP channel openers are working through, we should be able to look at trabecular meshwork cells and see if there's any sort of contraction or relaxation effect on those cells. And that's exactly what we see. Again, these are very preliminary results.

We have only done this on a couple of cell lines. But what you can see here is that here, we've taken trabecular meshwork cells. And this is actually done on a contraction assay, where we actually put a collagen gel in the bottom of a six-well Petri dish. Then you can layer cells over the top of that.

Once they grow up, then you can add in different agents and then actually measure to see if that collagen gel actually contracts or if it stays the same. And when you use LPA, which is a known contraction agent, you'll see a nice contraction of the gel. When we did that, you can see that these cells are contracting-- get nearly about a 20% change.

When you add in now the LPA plus the diazoxide, you can see that that inhibits that contraction. So it appears that diazoxide at least is inhibiting contraction. Whether or not it actually is involved in TM relaxation, that's hard to tell. But we do know that it's inhibiting contraction.

Another area that is somewhat debated in the literature is looking at the number of cells in individuals with primary open-angle glaucoma versus normal. And this is a study that was done back in 1984. And to make a long story short, what it shows is that individuals with primary open-angle glaucoma have less trabecular meshwork cells than age-matched controls, suggesting that something is going on with the cells. Either some of the cells are being lost due to apoptosis or some other mechanism.

So we looked at trying to determine if the KATP channels actually were protective, if they had a neural protection. And again, time's getting short. To make a long story short, the answer's yes. So when you add diazoxide, you'll actually see that it inhibits apoptosis of trabecular meshwork cells. So we still have a lot of work to do in regards to where these KATP channels are going from a physiologic standpoint.

This is one of the last slides before my conclusion. And what I want to mention here is that again, KATP channels may be neuroprotective. And these are some results that have been performed in other laboratories not our own, which have shown through a glutamate-induced exociticity or through ischemic retinal degeneration that when you add diazoxide to those particular cells, that you actually protect the cells from those types of insults.

So our next goals, our future goals, are twofold. One is to try to see if KATP channels can actually protect the retina. And as I mentioned, the only way to do this is to try to depict glaucoma. And so what we have to do is actually destroy the outflow pathway where we think this drug is working, but then treat the eyes and then look at the elevated intraocular pressure to see if the drug actually will protect the optic nerve to see if we'll have more retinal ganglion cells.

So that's one approach that we're looking for. The second approach is many of these compounds are only soluble in dimethyl sulfoxide and no one wants to put dimethyl sulfoxide on their eye. So we've been actually working with the University of Minnesota-- Peter Dosa at the University of Minnesota-- and he's actually been making us some pro drugs, some different compounds, based on the structure of diazoxide.

And we actually have a Minnesota Partnership Grant for this. And we've actually now found a couple compounds. We've identified a couple compounds that work very well, very similar to the parent, yet these now are only in aqueous solution. So we're moving closer to those drugs, but we have a lot of work to do on that. And we've got to move these into animal models.

So some take-home messages from today-- glaucoma's a neurodegenerative disease that affects over 60 million people worldwide. Elevated intraocular pressure's a causal factor and treatments are all geared towards reducing its levels. Again, the treatments out there right now are only involved in reducing elevated intraocular pressure. We're hoping that the drugs that we're looking at will also have protection for the retina.

The last three really are just that KATP channel opener's lower intraocular pressure through ex vivo and in vivo models by activating the ERK1/2 signaling pathway. These channels may be involved and they may be used to look at the physiology that's involved in regulating intraocular pressure homeostasis.

Again, there's very little known about what goes on and how fluid moves through the normal eye. These drugs allow us to look at that from a basic science standpoint. The offshoot of that which is extremely important is that KATP channel openers may have the potential to become a new class of therapeutics for the treatment of glaucoma. And that's where we're actually moving towards in the future.

So I'm going to leave you with this. Which sense do you fear losing the most? We know that we have five senses. There's touch, there's smell, there's taste, there's hearing, and of course, there's seeing.

In a poll that was done in 2010-- and I could ask all of you, if you had one of those senses, which is the one sense that you would fear losing, how many of you would say eyesight was your most important one that you'd fear losing? Yeah, just about all of you, and that's exactly what the poll said. About 82% said they'd fear losing their sight.

The next question was of those 82% of those people was how many of those people get routine checks. And 90% of them said no. So my last take-home message is if you're not getting routine eye checks, if you haven't done one for a while, please do that, especially if you get up to my age, which is getting in that danger zone.

So with that, I will stop and leave you with we wish you continued success in your research because this is kind of what it all feels like. So thank you very much. Thanks for your attention and have a good day.

[APPLAUSE]