

The Role of Adenosine in Epilepsy
CURE Epilepsy Webinar
(Transcript)

Priya Balasubramanian: 00:00

Welcome to our virtual seminar today. I'm Priya Balasubramanian, I'm Associate Director of Research at Cure Epilepsy and I want to thank you all for joining us today. Today is our first virtual seminar of 2021. This is part of our Frontiers in Research Seminar Series. Our seminar today is entitled The Role of Adenosine in Epilepsy, and this will discuss the anti-seizure and disease modifying effects of adenosine in epilepsy, as well as recent advances in developing adenosine kinase inhibitors as therapeutics for epilepsy.

Priya Balasubramanian: 00:37

The Frontiers in Research Seminar Series is generously supported by the Nussenbaum-Vogelstein Family. It aims to help educate and expose researchers, clinicians, and students to exciting epilepsy research, and also provide opportunities for young investigators to interact with leaders in the field. Lately we've been unable to provide this interaction live due to COVID-19. So until conditions allow us to all come back together in person, we will continue to present these seminars virtually in 2021. As you can see, our next virtual seminar is scheduled for March 12th, so mark your calendars. This will be on the use of organoids in epilepsy research and will be presented by Dr. Jack Perrin from the University of Michigan.

Priya Balasubramanian: 01:27

I also want to draw your attention to our post-doctoral data blitz seminar, which is scheduled for April 21st. If you're a postdoc with at least three years experience and if you're working on some exciting translational research in epilepsy that you'd like to present in this virtual format, you can email us your abstract and your biosketch to research@cureepilepsy.org if you'd like to be considered for a short presentation. The deadline for this submission is this Friday, the 26th, and you can find more details on our website as well.

Priya Balasubramanian: 02:09

As you all know, CURE Epilepsy has been proud to be a leader in the epilepsy research community for over 20 years and funded over 260 projects spanning 16 countries. We have three different funding mechanisms. The one highlighted on the slide is our Catalyst Award, which is our newest award, and it is intended to fund translational research in epilepsy. The key research priority areas include acquired and pediatric epilepsy, treatment resistant epilepsy, sleep and epilepsy, and sudden unexpected death in epilepsy or SUDEP.

Priya Balasubramanian: 02:49

All of our grant applications go through a letter of intent phase and a full proposal review by scientific reviewers as fellow members of our community who are touched by epilepsy. This

Catalyst Award, like I said, is intended to fund translational research and aims to advance new therapies into clinical application. The [RFA 00:03:12] for this award will be announced in Q2 this year. The CURE Epilepsy Award and the Taking Flight Award are underway and the Cure Epilepsy Award is open to established investigators and our Taking Flight Award supports junior researchers who have at least three years of post-doctoral expense

Priya Balasubramanian: 03:34

Today I'm very happy to welcome Dr. Detlev Boison. He is Vice Chair of Research and Training and Professor in the Department of Neurosurgery at Rutgers university. Dr. Boison's research seeks to translate fundamental mechanisms of biochemistry and energy metabolism into novel therapeutic approaches with the treatment of neurological conditions. Dr. Boison is a three-time CURE Epilepsy grantee, including a 2020 CURE Epilepsy Catalyst Award for his project; Adenosine Kinase Inhibitors for Anti-epileptogenic Therapy. I'm sure we'll get to hear some of that today.

Priya Balasubramanian: 04:12

Before Detlev begins, I'd like to encourage everyone to ask questions. You may submit these at any time during the presentation, you can type them into the Q & A tab located on the bottom of your Zoom panel and click send, and we'll do our best to get through as many as we can. The last thing I want to say is that our virtual seminar today, as well as all of our future seminars, they'll be recorded and they are available on our Cure Epilepsy website. With that, I'll turn it over to Dr. Boison. Thank you.

Dr. Detlev Boison: 04:47

Thank you very much, Priya, for this very kind introduction. I would also like to take the opportunity to specifically thank the CURE Foundation for unwavering support for almost 15 years now. What I'm presenting today is a journey starting with basic research findings leading to hopefully new therapeutics that can be used to prevent epilepsy. And this work wouldn't have been possible without the support of CURE right from the beginning and it's my great pleasure to take you onto the journey of the role of adenosine in epilepsy today.

Dr. Detlev Boison: 05:36

Why should we be interested in epilepsy? What I'm going to tell you today is based on an evolutionary principle, life probably started with adenosine. Smart people in the 1960s found out that the toxic gas, hydrogen cyanide can spontaneously form adenine. Adenine is nothing else, but H5C5N5. That's likely to assume that adenosine has been there at the beginning of life and if you look into cellular systems today you'll find the adenosine motive everywhere. So intracellularly, adenosine is

part of a lot of bio-molecules, of course, ATP, ADP, AMP, SAMSAH, NAD, FAD, coenzyme A, and of course RNA. Life started with RNA, not with DNA, and messenger RNA [inaudible].

Dr. Detlev Boison: 06:36 On the extracellular side, adenosine is a well-known agonist of the adenosine receptors. Adenosine is an important molecule in epilepsy because adenosine increases during a seizure, and it's the endogenous agent that normally stops seizure, which is based on activation of the adenosine A1 receptor.

Dr. Detlev Boison: 07:00 So if you want to construct a living system out of scratch, the most important thing you need to consider is energy homeostasis. And if you want to construct a living system out of scratch, you need a very simple system. The most simplistic system you can think of is whenever you have an energy crisis, you get a drop in ATP and ATP is cleaved to form more adenosine, and then select adenosine just as a global inhibitor to suppress everything that consumes energy. That's the underlying logic of the adenosine system. Whenever you have an energy crisis, lack a food, lack of oxygen, you end up like this guy, and what you think in a situation like this is caffeine, which is an adenosine receptor antagonist. So if we look at epilepsy from this principle, an epileptic seizure is just an excessive consumption of energy, which leads to a drop in ATP and an increase in adenosine. Adenosine is just there to save energy, and this can be achieved by stopping this seizure.

Dr. Detlev Boison: 08:19 As you know, there are a lot of challenges in more effective treatments for epilepsy. One third of all persons with epilepsy suffer from intractable pharmacoresistant seizures. There are variety of co-morbidities associated with epilepsy. There's a problem of SUDEP, and none of the currently available anti-seizure drugs have ever been designed to prevent or to modify the disease. So therefore there is a gap that needs to be filled. If you look into conventional anti-seizure drugs, they all have different targets. Most of them target ion channels, but they all have in common that they target directly epileptic hyperexcitability. But surprisingly, this principle doesn't work in one-third of all epilepsies.

Dr. Detlev Boison: 09:15 Another alternative is the use of metabolic therapies, which directly tap into biochemistry. The most important metabolic therapy is the ketogenic diet, which is a high-fat, low-carbohydrate diet, which is doing two things. On one hand, it leads to an increase in free fatty acids and the production of ketone bodies. And there's also a reduction in glucose. And together this mobilizes a lot of different pathways and

mechanisms, and one of them is an increase in the release of adenosine. So those metabolic therapies target directly energy homeostasis metabolism, and also epigenetic factors.

Dr. Detlev Boison: 10:09 Let's have a look into the biochemistry of temporal lobe epilepsy and the role of adenosine. First of all, it's important to make clear that the adenosine system is highly compartmentalized into an extra cellular component and intracellular component and an intranuclear component. Adenosine is derived mostly from the cleavage of ATP through a variety of different enzymatic pathways leading to adenosine. Then we have an equilibrative transport system that equilibrates extracellular and intracellular levels of adenosine. And on the the intracellular side, the key enzyme for the regulation of adenosine is adenosine kinase.

Dr. Detlev Boison: 10:56 What happens in a temporary lobe epilepsy is that there is a [inaudible 00:11:02] adaptive over-expression of adenosine kinase. And I refer specifically to the cytoplasmic isoform ADK-S. S stands for short, it's a little shorter this isoform. So if you increase the ADK-S, it phosphorylates adenosine into AMP, and this creates a gradient which drives the flux of extracellular adenosine inside the cell. And if you have less extracellular adenosine, the activation of the seizure suppressing adenosine A1 receptor is compromised, and this can be a direct cause for triggering epileptic seizures.

Dr. Detlev Boison: 11:46 But there's another important pathway which is taking place in the cell nucleus through the nuclear isoform ADK-L. So if ADK-L is increased, it removes adenosine in the cell nucleus and this drives the flux of methylation pathways through the transmethylation pathway. So transmethylation is the biochemical reaction through which a methyl group derive from S-Adenosyl methionine is transferred to an acceptor molecule, which in the cell nucleus can be DNA, which is a pathway that leads to increased DNA methylation. This pathway is dependent on the action of ADK-L, which drives the flux of methyl groups through this pathway and can lead to an increase in DNA methylation.

Dr. Detlev Boison: 12:47 Let's look first into the role of ADK-S, the cytoplasmic isoform, which through the equilibrative transport mechanism regulates extracellular adenosine. This is a study where we over-expressed ADK-S in a transgenic mouse model. And if we do, we create an adenosine deficient mouse model, which is characterized by spontaneous recurrent electrographic seizures, so these mice have around five seizures per hour. The phenotype of those mice is also characterized by a variety of

comorbidities commonly associated with epilepsy, for example, deficiency in working memory as shown here in a Morris water maze paradigm. That means over-expression of the cytoplasmic isoform ADK-S can trigger an epilepsy phenotype, which is associated with a variety of different comorbidities.

- Dr. Detlev Boison: 13:57 That means if we augment adenosine systemically in the extracellular space, we should be able to stop epileptic seizures. And we have shown this in a mouse model of pharmacoresistant epilepsy. This is a model where we inject kainic acid into the hippocampus of mice, which initially triggers a brief status epilepticus. And after a delay of a few weeks, these animals develop spontaneous recurrent electrographic seizures, which are resistant to conventional anti-seizure drugs, such as carbamazepine. However, if we give a systemic injection of an A1 receptor agonist, we can completely block those pharmacoresistance seizures. The same happens if we give an adenosine kinase inhibitor. This is kind of the model inhibitor of 5-ITU. If we give an adenosine kinase inhibitor we can likewise block those seizures. And if we combine 5-ITU with an adenosine A1 receptor antagonist DPCPX, seizure activity is restored, providing proof that seizure suppression by a 5-ITU is based on increasing extracellular adenosine acting on the A1 receptor.
- Dr. Detlev Boison: 15:19 This looks like a fantastic therapy. Why don't we use it for the treatment of epilepsy? The problem is, those systemic adenosine orientations therapies are associated with a sedative side effects which is probably acceptable for short-term dosage, but to treat someone over months and years with an agent like that would not be practical because of excessive sedation. That means we need to develop different strategies to supply adenosine to the brain. In the past, we have developed a variety of different cell-based or implant-based local adenosine delivery strategies.
- Dr. Detlev Boison: 16:09 But today, I want to talk more about epileptogenesis, which is the process that turns a healthy brain into an epileptic brain and the emerging role that adenosine plays in the control of epileptogenesis. Epileptogenesis can be triggered by a variety of acute brain insights, which could be traumatic brain injuries, stroke, brain infections, complex post-operative seizures. And this can create a cascade of different events that lead to a complete restructuring and rewiring of the brain, which after a delay of usually weeks in rodent models in months to years in humans, then leads to the onset of clinical epilepsy.

- Dr. Detlev Boison: 16:59 Among all those changes which have been identified, I want to focus today on glial changes and epigenetic reprogramming. So what we have shown, and this really goes back to initiative funding through the Cure Epilepsy Foundation, is the role of adenosine kinase in epilepsy and epileptogenesis. We've found that during epileptogenesis, there is a continuous adaptive increase in adenosine kinase, stent here in rat and in here in this [inaudible 00:17:45], and over-expression of adenosine kinase is linked to astrogliosis of the epileptogenic hippocampus. This process coincides with the development of spontaneous recurrent seizures.
- Dr. Detlev Boison: 18:03 We also find over-expression of adenosine kinase in human temporal lobe epilepsy, and over-expression of adenosine kinase in conjunction with astrogliosis can be considered a pathological hallmark of temporal lobe epilepsy. Of course those epilepsy models are very complex. There are a lot of different histopathological alterations in the epileptic brain. It's not just astrogliosis, it's also mossy fibers sprouting [inaudible 00:18:38] a variety of other changes.
- Dr. Detlev Boison: 18:40 In order to understand more clearly the role of astrogliosis and adenosine kinase for the generation of seizures during epileptogenesis, we need a more minimalistic model to study this process. We did this by creating a model of focal epilepsy, which is triggered by injecting a tiny dose of kainic acid into the amygdala, which triggers a focal onset that was epilepticus, which is terminated exactly 30 minutes later by lorazepam. As a result of this priming status epilepticus, we find the acute CA3 selective neuron injury. If we then wait three weeks, at the site of the original injury, we find local astrogliosis in conjunction with over-expression of adenosine kinase.
- Dr. Detlev Boison: 19:46 If we implant electrodes directly into this area, we can record spontaneous electrographic seizures. At that time I had various [inaudible 00:19:57] in my lab was, I was able to implant electrodes at different locations into the same hippocampus of the same mouse to record activity. As you can see here, electrographic seizure activity is exclusively limited to the CA3 area where we find astrogliosis and over-expression of adenosine kinase. But as soon as I injected an adenosine A1 receptor antagonists DPCPX, those seizures starting here immediately spread throughout the entire brain, which tells us that the adenosine system in the rest of the brain is still intact. And if the adenosine tone is removed by the A1 blocker, then the seizures can spread from this focal area.

- Dr. Detlev Boison: 20:54 The next step was to develop therapies to intervene with this process. The data I'm going to show now was basically the first indication that adenosine might have a noble role in the prevention of epilepsy. Although those data are not a complete proof, they provide a strong indication that adenosine might have previously unrealized anti-epileptogenic activity.
- Dr. Detlev Boison: 21:27 What we did in this experiment is we injected kainic acid into the amygdala to trigger the acute epilepsy triggering brain injury in the CA3, so you can see CA3 selective injury. And 24 hours later, we transplanted stem cells engineered to release adenosine based on an adenosine kinase knockout at the hippocampus, and those cells can integrate into the hippocampus formation, and then we waited three weeks. And three weeks later, we found a following. We found a statistically significant attenuation of astrogliosis, but adenosine kinase levers were almost completely normal.
- Dr. Detlev Boison: 22:18 So if adenosine kinase levels are not increased after the delivery of adenosine with stem surgery of brain implants, the prediction would be that we won't find epileptic seizures, and this is actually what we found. So in all our control enemas, we got an end result of around four seizures per hour after three weeks, however, in none of our enemas that received the knockout implants, we found any seizure in over 100 hours of EEG recordings. This is a first indication that adenosine might have anti-epileptogenic properties. However, at this time, we did not understand how this might work.
- Dr. Detlev Boison: 23:13 Let's have a look into epigenetics. Epigenetics are alterations to the genome. This can be DNA methylation or histone methylation, histone acetylation, non-coding RNAs. What has been found in a variety of different studies that there are changes in DNA methylation, which are associated with the development of epilepsy. If we look at DNA methylation, we immediately realize that we have a direct link to adenosine based on the transmethylation pathway. What happens is methyl groups are derived from S-adenosylmethionine and can be transferred to DNA. The end product S-adenosylhomocysteine is cleaved into adenosine and homocysteine by SAH hydrolase. And SAH hydrolase is an enzyme which has, well, where the thermodynamic equilibrium of the reactions on the side of SAH formation and SAH is a direct inhibitor of DNA methyltransferase.
- Dr. Detlev Boison: 24:38 In order to prove that change in adenosine kinase can actually affect Global 5-mC levers, we engineered Baby Hamster Kidney Fibroblasts to either a lack adenosine kinase, those are

knockout sets which has the lowest level of 5-mC. Then we used those knockout sets and introduced either ADK-S in the cytoplasm, which led to a small increase in 5-mC, or we over-expressed ADK-L, which is in the nucleus, which led to a massive increase in 5-mC. What basically happens if we increase adenosine kinase, we drive the flux of methyl groups through the transmethylation pathway, which then leads to increased DNA methylation. If this is true, then the opposite should also work. If we increase adenosine in the brain, we should be able to provide a block to this pathway and lead to a block in the DNA methylation.

Dr. Detlev Boison: 25:57 The strategy that we chose was in a collaboration with David Kaplan from Tufts to engineer the [biopolymer silk 00:26:06] to release adenosine. And the advantage of using silk is that we can precisely define the release kinetics of adenosine and the release duration. Those implants were designed to release define dose of adenosine for a restricted time span of only to 10 days. If we increase adenosine, we can shift the equilibrium of this reaction towards the formation of SAH, and SAH is an inhibitor of DNA methyltransferase. This should be a strategy to reduce global DNA methylation.

Dr. Detlev Boison: 26:46 We tested this in the rat systemic kainic acid model of epilepsy. And in this study, we injected kainic acid to prem the epileptogenic process, but we waited until nine weeks, until all animals had developed the first set of epileptic seizures. We started basically treatment after the diagnosis of clinical epilepsy in these animals. And it's important that in this animal model, epilepsy is progressive. So over time, seizures get more and more and more severe. We took these early epilepsy rats, and then implanted the adenosine released in polymers at a week nine and they released adenosine for only 10 days and we monitored these animals for a total of three months.

Dr. Detlev Boison: 27:41 What we found is that adenosine normalizes DNA methylation. What we find at the nine-week time point prior to the polymer implantation, we find increased activity of DNA methyltransferase. However, during active adenosine release from the polymers, DMMT activity was almost completely blocked. So when we quantified 5-mC, before polymer implantation we already find high methylation of DNA. During active adenosine released, DNA methylation levels are reversed back to normal. And importantly, this was maintained even four weeks after cessation of adenosine release from the polymers.

Dr. Detlev Boison: 28:36 Now we looked at seizure development in those rats. And what you can see here is all the animals had developed the first set of

seizures prior to implantation of the polymer. In all control rats, epilepsy continued to increase over time. Here is the group that received trends in adenosine. And during active adenosine release marked by this red bar, seizure activity is almost down to zero. That means the local delivery of adenosine provided almost complete suppression of seizure activity. But afterwards, there was no further increase in seizure activity compared to the baseline. Those three data points are still a little bit low which might not be due to some lingering washout effects from adenosine from the polymer. But if you compare the data that reach up to three months after polymer implantation, they are pretty much at the same level as the pre-implantation baseline.

Dr. Detlev Boison: 29:55 So with this study, we were able to block the progression of epilepsy development, which has also independently been validated by a suppression of mossy fibers sprout, which is a pathological hallmark of the epileptic brain. This is the degree of mossy fibers sprouting prior to polymer implantation. And the black bar is the adenosine group in which we were able to completely prevent any progression in mossy fiber sprout, which is an independent experiment without an addition to those seizure data.

Dr. Detlev Boison: 30:40 Those data show that adenosine augmentation can actually prevent the progression of epilepsy with its most sophisticated studies that [inaudible 00:30:53] experiments to look at genes that changed during epilepsy development and which are restored back to normal with its pacified sequencing and came up with, here we go, came up with a list of target genes, and we found among those genes that are regulated, genes that are active in the cell nucleus. We found polymerases, zinc-finger proteins, in general factors that interact with DNA gene transcription and translation which might be a homeostatic master regulatory system to regulate the entire genome and methylome on a higher level.

Dr. Detlev Boison: 31:46 This brings us back to the adenosine kinase hypothesis of epileptogenesis which happens after an acute trigger for its [inaudible 00:31:55] epilepticus. We get astrogliosis, over-expression of adenosine kinase, and a progressive decrease in the tone of adenosine during epileptogenesis. The deficiency in adenosine specifically in the nucleus increase DNA methylation, which is needed for the maintenance and progression of epilepsy. And by employing an adenosine therapy, we are able to interrupt this vicious cycle and prevent the progression of epilepsy. That means obviously adenosine kinase is a suitable target for drug development. So while implanting adenosine releasing might not be the most practical approach for the

treatment of human patients at risk for developing epilepsy, a small molecule inhibitor might be more versatile and could be used prophylactically or sufficiently safe.

- Dr. Detlev Boison: 32:58 So adenosine kinase is a very interesting protein. It's a relatively small protein encoded by 11 exons, with the coding sequence of only 1.1 kilo bases, but the gene is monstrous. It's over 500,000 base pairs in humans, and it takes eight hours to transcribe. So the role of adenosine kinase is it catalyzes the reaction. It takes a phosphate group from ATP, transfers the phosphate group to adenosine forming AMP and ADP. It's important to realize that we have these two forms of adenosine kinase with ADK-S in the cytoplasm regulating the tissue tone of adenosine and adenosine receptor activation and ADK-L in the cell nucleus has autonomous effects, specifically epigenetic effects which form the basis for the anti-epileptic potential of adenosine.
- Dr. Detlev Boison: 34:04 The therapeutic goals would be to develop a novel set of ADK inhibitors with a proven activity on ADK-L in the cell nucleus. To provide a proof of principle that small molecule ADK inhibitors can prevent epilepsy, we used the, and this was also a Cure funded study, We used the global ADK inhibitor 5-ITU in the [intrahypocapokainic 00:34:37 acid] model of epilepsy.
- Dr. Detlev Boison: 34:39 In this study, we waited three days after priming epileptogenesis with kainic acid and injected these animals for five days only with 5-ITU, and then monitored the seizure phenotype at six and nine weeks. What we found is the following, that in 50% of all animals, we were able to completely prevent epilepsy development. This is quite a significant success. And the reason why there is a little variability is likely the time window. So we are planning to extend the time window a little further up to 10 days. We try to match the time window precisely to histopathological changes that we see in the epileptic brain. But importantly also, suppression of epilepsy in the ITU treated group was also associated with the prevention of granular [inaudible 00:35:53].
- Dr. Detlev Boison: 35:53 With this study, we were able to show that it is absolutely feasible to prevent epilepsy with the trends in those of an adenosine kinase inhibitor. Therefore, and this is now funded very generously through CURE and the Catalyst Award, we move on in developing a new set of adenosine kinase inhibitors, and that's a collaborative project with Dr. Ken Jacobson, who was an intramural NIH scientist. He spent his entire life working on the development of adenosine receptor agonists and antagonists, and he knows the adenosine medicinal chemistry in and out. He is currently Adenosine A3 agonists in phase three

clinical trials. What we did is to generate a new set of novel adenosine kinase inhibitors. What we employ is a chemical trick.

- Dr. Detlev Boison: 37:04 Normally, ribose has an oxygen here, and the oxygen allows the ribose to shift confirmation and to wobble around between a south and a north configuration. But by removing the oxygen and introducing a tri-cyclic carbon ring here, we can fix the ribose either south or north configuration. We propose that these stereo form of the ribose is essential for driving specificity for either the cytoplasmic or the nuclear as a form of adenosine kinase.
- Dr. Detlev Boison: 37:42 What you can see here is we looked at IC50 data from a set of a variety of new compounds. And what you can see is that most of those new compounds have IC50s that are much lower than the reference compound 5-ITU. One of our compounds MRS4203 is currently our lead compound, which we [inaudible 00:38:15]. What we have seen so far is data from an epigenetic drug screening platform. So it's important to really validate that those new adenosine kinase inhibitors can affect DNA methylation and ADK-L dependent manner. And we do this by using [BH 00:38:40] cases that either lack adenosine kinase or are engineered to only express ADK-L.
- Dr. Detlev Boison: 38:51 As you can see here, ADK-S's are hyper-methylated compared to the knockouts, the ADK-S's of ADK in the nucleus and ADK-S's of ADK in the cytoplasm. Then we use this lab-based screening platform to screen our adenosine kinase inhibitors. And what you can see here is that our reference component 5-ITU reduces 5-mC levels in the ADK-S's compared to the vehicle. And the same is true and much better for our lead compound MRS4203, which is in the south configuration. And the sister molecule NRS4380, which is almost the same molecule, but in the North configuration does not have this activity.
- Dr. Detlev Boison: 39:54 We also ran those compounds in ADK knockout cells to screen for off target effects. And what you can see here is that conventional adenosine kinase inhibitors have off target effects, but those two new ADK innovators don't have any activity in the ADK-null cells. We were also able to show that MRS4203 blocks DNA methyltransferase activity in the brain, leads to reduction in hippocampus 5-mC, which is superior to a 5-ITU. We also looked for side effects in the open field and there is, 5-ITU has sedative side effects which must much less profound in the MRS4203 [inaudible 00:40:53].
- Dr. Detlev Boison: 40:53 Our goal is to develop our medicinal chemistry projects further to develop new compounds that can be developed into

therapies for the prevention of epilepsy. With this, I would like to thank all of you for your attention and I wish to thank the Cure Foundation for inviting me and for support over almost 15 years. You have really been part of the story. I wish to thank my fabulous research team, my collaborators, and this was one of the last photos we were able to take before we were all forced to wearing masks. So thank you very much again and I'm happy to take any questions.

Priya Balasubramanian: 41:50

Thank you so much, Detlev. That was a great talk. We have some time for questions. I just wanted to remind everyone in the audience, if you have questions, please submit them through the Q & A tab and just click send. We can start off with a couple questions that have come in. The first question is have you observed or looked into DNA methylation differences in [inaudible 00:42:17] in response to adenosine?

Dr. Detlev Boison: 42:18

That's a good question. We have primarily seen changes in neurons. Those are new data which I haven't shown today, but we find the trends in ectopic expression of ADK-L in neurons during epileptogenesis. This is really a transient period and might be the reason why trends in treatment with an ADK inhibitor perfectly tailored to this trends in over-expression of ADK-L in neurons which really coincides with increased 5-mC during the same time span might be an important role for the epileptogenic process.

Priya Balasubramanian: 43:17

Thank you. Then we have another question here. He said: I was wondering if the medications change the seizure onset or property of the seizure in a long time. So over time, do the medications change the seizure onset or property of the seizures?

Dr. Detlev Boison: 43:39

The goal here is basically to have a prophylactic treatment to initiate, and yet to have trends in treatment during the latent period of epileptogenesis with the ultimate goal to prevent epilepsy all together. Now in most studies, which I'd shown previously, we compared the seizure phenotype at six weeks and nine weeks after initiating epilepsy and we did not find any differences. Of course, it would be worthwhile in future studies to extend that time period to longer time spent looking at brains at three months or six months and we will certainly do this in the future.

Priya Balasubramanian: 44:37

Thank you. Another question that's come in is they are asking if there are any commercial assays available for measuring adenosine levels in human plasma.

Dr. Detlev Boison: 44:49 No, there's not. Adenosine can be quantified by HPLC and by LCMS/MS methods. There are no commercial assays. And the blood-brain barrier is not really penetrable for adenosine and adenosine in the circulation has an extremely sharp half-life in the range of seconds. So plasma adenosine is unlikely to be a representative for adenosine levels in the brain.

Priya Balasubramanian: 45:21 That makes sense. The next question is how has astrogliosis linked with epilepsy? Do we know if it causes epilepsy or is it just correlated with epileptic seizures?

Dr. Detlev Boison: 45:37 There's a lot of data, not just from my group, but also from other researchers that suggests that astrogliosis can truly be a cost for the generation of epileptic seizures. And astrogliosis is triggered by all the inflammatory processes that trigger the initial phases of epileptogenesis. So you get microglial activation and astroglial activation as a response basically to brain inflammation.

Priya Balasubramanian: 46:19 Okay. Then how specific is 5-ITU for ADK?

Dr. Detlev Boison: 46:30 It's one of the old inhibitors. It's not selected for ADK-L versus ADK-S. According to our findings, it blocks both. That's the reason why it prevents epileptogenesis, but it also has sedative side effects because it also increases extracellular adenosine.

Priya Balasubramanian: 46:51 Thank you. Do you know of a mechanism by which adenosine may build up in the nucleus and make methylation? Would you like me to repeat that?

Dr. Detlev Boison: 47:16 Yeah.

Priya Balasubramanian: 47:16 They're asking if you know the mechanism by which adenosine could build up in the nucleus and lead to methylation.

Dr. Detlev Boison: 47:26 Yeah. The deficiency of adenosine in the nucleus would drive DNA methylation. If there's a mechanism that increases adenosine in the nucleus, then it would block DNA methylation and this could be directly linked to factors that inhibit adenosine kinase in the nucleus. But this is currently unknown.

Priya Balasubramanian: 47:53 Okay. I don't see any more questions coming in. I'll just give it a second here. If there's any more questions, please type them in the Q & A tab. While we're waiting, Oh, I see something here. Here we go. This is interesting, how did you go about designing a biopolymer that could release adenosine?

Dr. Detlev Boison: 48:32 That's an interesting question. Whenever you have a release system, you get a logarithmic release profile. If you, let's say, just encapsulate adenosine in the membrane, you will have a burst release: Most of it will come out immediately and then drop off. So in order to get a stable release, you basically need to combine several logarithmic release profiles in one implant. So the way those silk polymers were engineered was: First we encapsulated adenosine as micro-vesicles in a silk membrane, and then those micro-vesicles were embedded in a 3D silk metrics. Then the whole construct was coated with alternative layers of adenosine and silk, always repeating, and then you can cap the whole thing with several additional layers of silk to slow down the release. So if you do it right, then you get the release profile.

Priya Balasubramanian: 49:50 Very interesting.

Dr. Detlev Boison: 49:52 And the beauty of silk is it's a biopolymer. It doesn't have any [inaudible 00:50:02], no toxins. It's fully resolvable. Silk has been used as a suture material for decades and it's a relatively boring amino acid repeat of glycine and alanine. So it's very biocompatible.

Priya Balasubramanian: 50:27 Excellent. We have a question about what are good targets for DNA methylation to measure?

Dr. Detlev Boison: 50:40 Good targets for DNA methylation to measure. We are primarily interested just in the global DNA methylome, because based on the evolutionary principles, I think this is a primordial mechanism to regulate the entire DNA methylome. Because if you think in terms of evolution, if you want to regulate genes, you cannot start with transcription factors because transcription factors need their own genes, they need to be controlled by protein kinase pathway, which all needs their own genes which need to be regulated. This is way too complicated.

Dr. Detlev Boison: 51:31 Now, if you really want to develop a regulatory system for the genome, the most simple way you can think of is just adding and removing methyl groups. This affects the entire genome and I believe this was probably one of the first mechanisms that came up in evolution to regulate the entire DNA methylome on a global level, and everything else that creates target specificity was invented later during the evolution.

Priya Balasubramanian: 52:21 Thank you. There is a question about your MRS drug and whether it is BBB permeant: Does it permeate the blood-brain barrier?

- Dr. Detlev Boison: 52:33 Yeah, we have evidence that it goes through the blood-brain barrier. We've also engineered ADK-L mice, which over-express ADK-L in the brain and we can find effects of MRS4203 in those ADK-L mice which is a direct proof that it has an effect in the brain.
- Priya Balasubramanian: 52:55 Mm-hmm (affirmative). There's a non-technical question about whether there's any application of your research yet for treating patients who have intractable epilepsy.
- Dr. Detlev Boison: 53:14 That's a good question. The primary goal right now with those new compounds is epilepsy prevention, to prevent them from becoming intractable. To treat intractable patients with adenosine is also doable. We have shown that adenosine can prevent pharmacoresistant seizures in our epilepsy models. The challenges are for intractable patients we need long-term therapies over months or years, which means the drugs we have to use need to be very, very safe and we need to come up with solutions to avoid side-effects based on extracellular adenosine. One way to achieve those goals would be focal therapies. One approach could be gene therapy to knock down adenosine kinase in an epileptogenic brain area, but that's not all that quick solution most likely.
- Priya Balasubramanian: 54:40 Right. I think we have time for a couple more questions. There's one about the role, what is the role of AMP in epileptogenesis? Are some of the effects of adenosine kinase due to increase of AMP?
- Dr. Detlev Boison: 55:00 Most likely not because AMP levels in cells are 100,000 fold higher than adenosine. So if you change adenosine kinase, you have dramatic effects on the levels of adenosine without changing the levels of AMP significantly.
- Priya Balasubramanian: 55:23 Okay. And final question here. They're asking while A1R seem to have the dominant and inhibitory effect, what roles do other adenosine receptor subtypes have on seizures, particularly in the cortex, as opposed to the hippocampus?
- Dr. Detlev Boison: 55:43 That's a very good question. The answer to this question is that we need to realize that the adenosine system is highly compartmentalized. There is one kind of global tissue tone of adenosine, or which also can be considered as an extracellular compartment, which is directly under the control of adenosine kinase expressed in astrocytes and which provides tonic inhibition through activation of the A1 receptors. This is just a global tissue tone of adenosine that primarily activates the A1 receptor to provide the tonic inhibition in the brain.

Dr. Detlev Boison: 56:29 But on top of that, there is also a synaptic pool of adenosine. So neurons under high-frequency stimulation can directly release adenosine, and neurons can release ATP, which is rapidly broken down to adenosine. So there is a different source of adenosine at the synaptic level. And the A to A receptor has primarily a role to find you the activity of adenosine on the synaptic level. The rationale of this mechanism is basically the forearm. So if you have a globally inhibited networks through the A1 receptor, and then you have once synapse where you have adenosine providing activation of A to A receptors, you can improve the signal to noise ratio. You can even more specific signal on the synaptic level in a globally inhibited network.

Priya Balasubramanian: 57:43 Thank you so much, Detlev. Finally there's a comment, it's not a question, but just a comment to say congratulations and thank you for your work.

Dr. Detlev Boison: 57:52 Thank you.

Priya Balasubramanian: 57:54 Definitely echo there. And with that, we can conclude our virtual seminar. I want to say thank you to you, Detlev, again for your presentation and I also want to thank the Nussenbaum-Vogelstein Family again for their generous support of this seminar series. Thank you all in the audience for asking questions and being engaged. And if you'd like more information, please you can write to us, you can visit our core researchers page, or you can write to us at research@cureepilepsy.org. Make sure you register for our next seminar on March 12th. There's a brief survey after this webinar, please do take that survey and we value your feedback. Thank you all, and stay safe.