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Diabetes Mellitus and Hypercholesterolemia are Risk Factors for Alzheimer's Disease and Appear to Affect the Integrity of the Blood Brain Barrier

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Abstract:

Studies have shown that the vascular risk factors common to diabetes mellitus and hypercholesterolemia are also risk factors for Alzheimer's disease (AD). It is currently unknown how these diseases are associated with AD, but they may cause a leak in the blood brain barrier (BBB), which is one of the hallmarks of AD. In this preliminary study, over 150 pig brain slides were tested for the expression levels of tight junction proteins occludin and claudin V in the BBB microvasculature. There were three groups of pig brains used in this study namely, control pigs, pigs with diabetes mellitus and hypercholesterolemia (DMHC), and DMHC pigs treated with darapladib, a drug being developed by GlaxoSmithKline for the treatment of atherosclerosis. The results showed changes in the level of expression of occludin and claudin V in the DMHC treated pigs compared to control pigs. The results also revealed expressional changes of claudin V and occludin in the drug treated pigs compared to control pigs. Therefore, these results appear to suggest that long-term diabetes mellitus and hypercholesterolemia conditions may have an effect on the expression of tight junction proteins found in the BBB. This may provide a plausible explanation for the observed increase in BBB permeability in DMHC pigs and also a mechanistic link that may explain why diabetes is now considered a major risk factor for AD.

Chapter 1

Introduction:

1.1 Alzheimer's Disease (AD) Background

Alzheimer's disease (AD) is a neurodegenerative disease associated with memory loss, cognitive decline, a loss of the ability to carry out everyday tasks, and eventually death. This is caused by the death/damage of neurons and loss of the synaptic connection between them, especially between the cholinergic neurons throughout the brain. The rate of incidence in people over the age of 85 is nearly 40%. The recent rapid increase in incidence of this disease has much to do with the increase in our average lifespan. The destruction of these neurons and the overall cause of this disease remains unknown but many theories have been postulated to explain the pathogenesis of AD. The only unambiguous aspect associated with AD is the build up of amyloid plaques and neurofibrillary tangles in the brains of AD patients. ("Adni," 2010)

1.2 Theories as to the cause of AD

Many different views have arisen about the cause of AD. One theory is that Abeta 42 is able to enter into the brain via a leaky BBB and attach to neurons. Originally it was believed that amyloid plaques began accumulating outside of the neuron and the neurons would die because of the plaques impinging on their space. In an interview with Dr. Nagele (2010) he proposes a new theory such that the amyloid

peptide (especially Abeta 42) accumulates inside the neuron and the neuron dies because of the build-up of indigestible Abeta 42. After the neuron dies all that is left behind is an amyloid plaque. The continuous destruction of neurons and loss of synaptic connections prior to and during this process causes an AD patient to eventually die.

The death of neurons is believed to occur from Abeta 42 entering the brain. Abeta 42 is a peptide made up of 42 amino acids. This Abeta 42 is the main constituent of the amyloid found in AD patient's brains within neurons and amyloid plaques. Though the neurons also synthesize Abeta 42, the amount of Abeta 42 generated in general circulation is several-fold higher than that in the cerebrospinal fluid that bathes the neurons. As the concentration of Abeta 42 is higher in the general circulation, any breach in the BBB can cause leakage of Abeta 42 into brain, which then acts to raise the overall concentration of Abeta 42 in the CSF which is in contact with the neurons. Under normal physiological conditions, the intact BBB is able to keep the circulating blood and its plasma components separate from the brain tissue parenchyma. It is able to do this because the capillaries in the brain contain a monolayer of specialized endothelial cells that line the lumen of blood vessels and also form tight junctions with each other so that the molecules cannot seep from the blood into the brain in response to a pressure gradient. Thus, the BBB prevents molecules in the blood from escaping into the brain. If there is a leak in the tight junctions between the capillary endothelial cells then the molecules, which normally remain in the blood can and will enter into the brain. (Clifford et al., 2007)

The BBB appears to be disrupted in many different neurodegenerative diseases. Preliminary studies have also suggested that the BBB is disrupted when Lipoprotein-associated phospholipase A2 (LP-PLA_2), which is a phospholipase found circulating in our blood, becomes irritated due to oxidative stress. When the LP-PLA_2 is disrupted it will release a part of its attached lipid as a fragment called LPC, lysophosphatidylcholine. This LPC is apparently an irritant that interacts with cell membranes, including vascular endothelial cells that line the blood vessels, and irritates them. Specifically, the endothelial cells that line the BBB will be irritated when LPC is released. If the endothelial cells in the BBB are irritated, we propose that this stimulates them to contract, thus causing tractional forces to be applied to the tight junctions forming the BBB, which are not able to withstand such tractional stress. The result is either transient or a more permanent leak, depending on the nature and duration of the insult. (Nagele, 2010) If the BBB remains intact, then the blood-borne Abeta 42 peptides will remain within the blood. But, if there is a BBB leak, the Abeta 42 will enter into the brain and bind selectively to the alpha 7 nicotinic acetylcholine receptors on a certain subset of neurons (mostly pyramidal neurons) in the brain tissue. (Clifford et al., 2007)

1.3 The Role of Abeta 42 in AD

Studies have shown that Abeta 42 binds to alpha 7 nicotinic acetylcholine receptors (a7nAChRs) with high affinity. AD patient brains show a decrease in nAChRs, including the a7nAChRs. A7nAChRs "modulate Ca+ ion homeostasis and the release of acetylcholine" (Nagele, D'Andrea, Anderson, & Wang,

2002). If there is a decrease in a7nAChRs then these functions will be disrupted causing some of the symptoms of AD, such as "cognitive dysfunctions" (Nagele, D'Andrea, Anderson, & Wang, 2002). A7nAChRs are found in high quantities in the hippocampus and cortex portions of the brain. Abeta 42 and a7nAChRs have a very high binding affinity. The a7nAChRs are located on the neuron surface so, when Abeta 42 enters the brain tissue through a leaky BBB, it will readily bind to a7nAChRs. This strong binding between the receptor and Abeta 42, may explain why the Abeta 42 is readily found on the surface of neurons and also why AD pathology primarily affects cholinergic and cholinoceptive neurons and is considered a synaptic loss disease. (Nagele, D'Andrea, Anderson, & Wang, 2002)

Abeta 42 cannot readily enter into the neuron on its own after binding to a7nAChRs. It appears that Abeta 42 enters the neuron with the help of brain-binding autoantibodies (Levin et al., 2010). There are certain brain-reactive autoantibodies in a person's blood that can bind to the surfaces of neurons. Studies done by Dr. Nagele and his lab suggest that without these autoantibodies, most of the cell-surface-bound Abeta 42 would not be able to enter the neuron as readily. However, in presence of autoantibodies that enter into the brain from the blood through a breach in the BBB, the amount of Abeta 42 entering the neuron becomes very high. It has been proposed that the binding of autoantibodies to the surfaces of vulnerable neurons induces receptor-mediated endocytosis, and that this autoantibody binding is the driving force behind Abeta42 internalization in neurons (Levin et

al., 2010) Since Abeta 42 that reaches the lysosomal compartment is indigestible to the neuron, the Abeta 42 will continue to accumulate in the neuron causing the neuron to eventually die and rupture. The death of the neuron will also cause any remaining synapses to die. All that remains is the remnants of neurons and their content of non-degradable Abeta 42 in the form of amyloid plaques. (Clifford et al., 2007)

This destruction of the neuron will also leave behind other cellular remnants, including various cytoplasmic proteins and residual DNA from the nucleus. (Nagele, D'Andrea, Anderson, & Wang, 2002)

1.4 THE ROLE OF THE BLOOD BRAIN BARRIER IN AD

It is important to understand how and why the BBB acquires leaks. If leaks can be prevented in the BBB then it may be possible to slow down AD progression or even prevent it. If it is the leaky BBB that allows the Abeta 42 and the autoantibodies to come in, then preventing these leaks would prevent the entry of these proteins (Clifford et al., 2007). The BBB is now considered to be primarily a network of endothelial cells held together by tight junction proteins such as claudin V and occludin. This barrier protects against the passage of many plasma components into the brain cerebrospinal fluid. It selectively allows some small molecules in such as O_2 and CO_2. It is only when there is a leak in between these endothelial cells that Abeta 42 and autoantibodies can enter. Studies have shown that diabetes mellitus and hypercholesterolemia may cause leaks in the BBB. Diabetes mellitus and

hypercholesterolemia appear to increase a person's risk of developing AD, but why it does this is still unclear. It is likely that these conditions cause a breakdown of the BBB.

Chapter 2

Rationale

It is important to understand what causes the leaks in the blood brain barrier (BBB). Preventing the breakdown of the BBB may prevent most cases of AD and may have a comparable beneficial effect on other neurodegenerative diseases. Diabetes mellitus and hypercholesterolemia (DMHC) may cause a breach in the BBB; therefore, making a person more susceptible to AD. This theory was tested by using a DMHC pig model, which has the virtue of a comparable vascular hierarchy similar to humans, and can be studied by using immunohistochemistry technique on brain sections.

Chapter 3

Materials and methods:

3.1 PORCINE MODEL

Full experimental details of the DMHC porcine study have been published previously (Wilensky, et al., 2008). Briefly, one month after DMHC induction, pigs were randomly assigned to either a control group or a treatment group receiving 10 mg/kg/d orally of the selective Lp-PLA2 inhibitor darapladib (SB480848, GlaxoSmithKline). Three pigs did not undergo DMHC induction and acted as age-matched controls. Pigs were sacrificed 28 weeks after DMHC induction (that is, 24 weeks after initiation of treatment). The selectivity of darapladib for human Lp-PLA2 has been reported previously (Wilensky, et al., 2008). Investigators were blinded to the group allocation. Not all of the original animals had brain specimens removed because of healthy behavioral status observed at mid-study using a staggered protocol. Specimens from the brains of 28 pigs were collected which included 13 (from a total of 17) DMHC untreated and 12 (from 20) DMHC treated with darapladib, as well as 3 untreated normal controls. Importantly, these animals were representative of the whole group given their near identical end of study measures for plasma cholesterol, glucose and Lp-PLA2 activity to those previously published (Wilensky, et al., 2008). For the animals included in the current analysis, the end of study plasma cholesterol and glucose values (average + SD) for the DMHC control and DMHC darapladib groups were 613 + 331, 699 + 302 and 382 + 110, 369 + 99 mg/dl, respectively.

The end of study plasma Lp-PLA2 activity (average + SD) values for the DMHC control and DMHC darapladib groups were 88 + 28 and 12 + 10 nmol/min/ml, respectively. (Acharya, et al., 2012)

3.2 PREPARATION OF PIG BRAIN TISSUE FOR MORPHOLOGICAL ANALYSES

Each brain was subdivided anteroposteriorly into regions designated anterior, middle and posterior and prepared for routine paraffin embedding and histological examination. For each of these brain regions, our analyses were focused on the cerebral cortex, since this layer retains a nearly identical cytoarchitecture throughout the brain and thus facilitates direct comparison of one brain region to the next. Each brain specimen was sectioned in a way to maximize the amount of tissue available for quantification. Total areas of cortical and non-cortical brain tissue in each tissue section were measured in hematoxylin and eosin-stained sections using image analysis and Image Pro Plus software described below. (Acharya, et al., 2012)

3.3 ANTIBODIES

The following antibodies were used for treatments of pig brain slice cultures. Mouse anti-claudin V was obtained from Invitrogen (Carlsbad, California) (monoclonal, cat. No. 35-2500, dilution 1:100). Rabbit anti-occludin was obtained from Invitrogen (Carlsbad, California) (polyclonal, cat. No. 71-1500, dilution 1:100).

3.4 IMMUNOHISTOCHEMISTRY

Immunohistochemistry was carried out using paraffin-embedded pig brain tissues as previously described. Briefly, tissues were deparaffinized using xylene and rehydrated through a graded series of decreasing concentrations of ethanol. Antigenicity was enhanced by microwaving sections in citrate buffer. Endogenous peroxidase was quenched by treating sections with 0.3% H_2O_2 for 30 min. Sections were incubated in blocking serum and then treated with primary antibodies at appropriate dilutions for 1 hr at room temperature. After a thorough rinse in PBS, biotin-labeled secondary antibody was applied for 30 min. Sections were treated with the avidin-peroxidase complex (Vectastain ABC Elite, Vector Laboratories, Inc., Foster City, CA) and visualized with 3-3-diaminobenzidine-4-HCL (DAB)/ H_2O_2 (Imm-Pact-DAB) (Vector). Sections were then lightly counterstained with hematoxylin, dehydrated through increasing concentrations of ethanol, cleared in xylene and mounted in Permount. Specimens were examined and photographed with a Nikon FXA microscope, and digital images were recorded using a Nikon DXM1200F digital camera and processed and analyzed using Image Pro Plus (Phase 3 Imaging, Glen Mills, and PA) and Cell Profiler image analysis softwares.

3.5 IMAGE ANALYSIS AND STATISTICS

The amount of claudin V and occludin found in the pig's BBB vessels was quantified using immunohistochemistry. Five images from each tissue section were recorded under identical illumination, magnification and camera settings using a Nikon FXA microscope equipped with a Nikon CCD camera. An image analysis software called Cell Profiler was used to quantify the total amounts of positive

stained claudin V and occludin. The total amount of claudin V and occludin-positive material in each viewing field was measured by pixel counting using Cell Profiler to determine the percentage of the total viewing field occupied by claudin V and occludin-immunopositive material. In addition, we also used Cell Profiler to count the number of occludin and claudin V-positive stained vessels in the same field. All data were downloaded into Excel Spreadsheets. The relative amount of claudin V and occludin per claudin V- and occludin-immunopositive stained specimens among the different treatment groups was calculated. Data is presented in graphs as mean amount of occludin and claudin V per claudin V and occludin-positive vessel along with the standard error. A t test was used to test the significance of differences among the different treatment groups.

Chapter 4

Experimental results

4.1. Selection of Pig Brain Sections

Pig brain slices were taken and mounted onto slides for use in immunohistochemistry. A large sample size was used in an effort to get accurate counts for the control, drug treated, and DMHC pigs. After immunohistochemistry was performed on the slides five pictures of random viewing fields were taken of each slide, and they were all processed through cell profiler using a specific pipeline, which is described above in the materials and methods section. The pig model was used to test how diabetes mellitus and hypercholesterolemia (DMHC) affect the BBB, which was done by observing expression levels of claudin V and occludin. Figure1 shows one section of a particular slide immunostained for both occludin and claudin V. Cell profiler was able to easily distinguish the positive stained vessels from the background due to the deep brown color. This allowed for accurate counts for each brain section in all three categories.

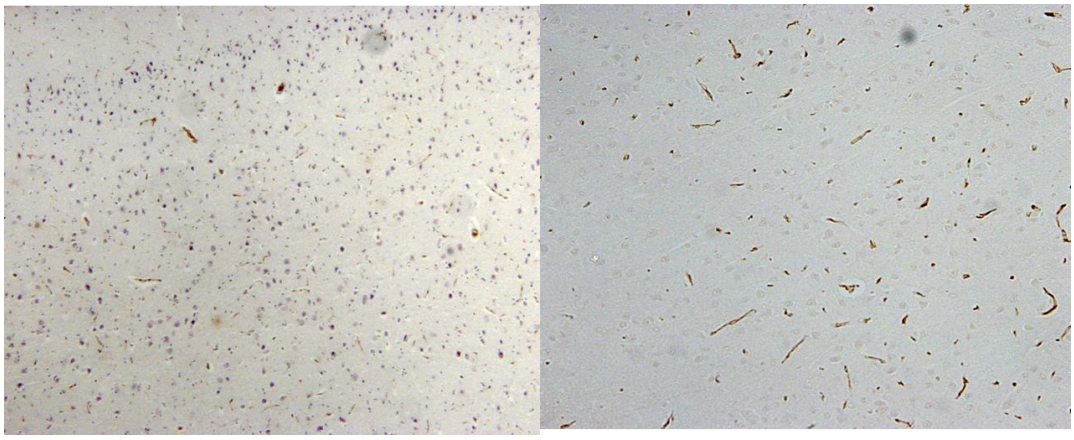

Fig.1. Images of occludin (left) and claudin V (right) taken at 4X that were used in cell profiler processing. The positively stained vessels appear brown in the images making it easy for cell profiler to pick it up out of the image and count.

4.2. CLAUDIN AND OCCLUDIN'S ROLE IN THE BLOOD BRAIN BARRIER

In Figure 2 claudin and occludin interact with one another on adjacent endothelial cells to help form a tight seal, which prevents unwanted molecules from entering. If the integrity of the tight junctions is altered then the BBB is breached and unwanted molecules from the plasma are able to enter into the brain. Diabetes mellitus and hypercholesterolemia are believed to compromise the integrity of these tight junctions, which may be the reason these conditions are a risk factor for AD. Diabetes mellitus and hypercholesterolemia do affect the BBB tight junction proteins claudin V and occludin by causing them to be over expressed in most regions of the brain.

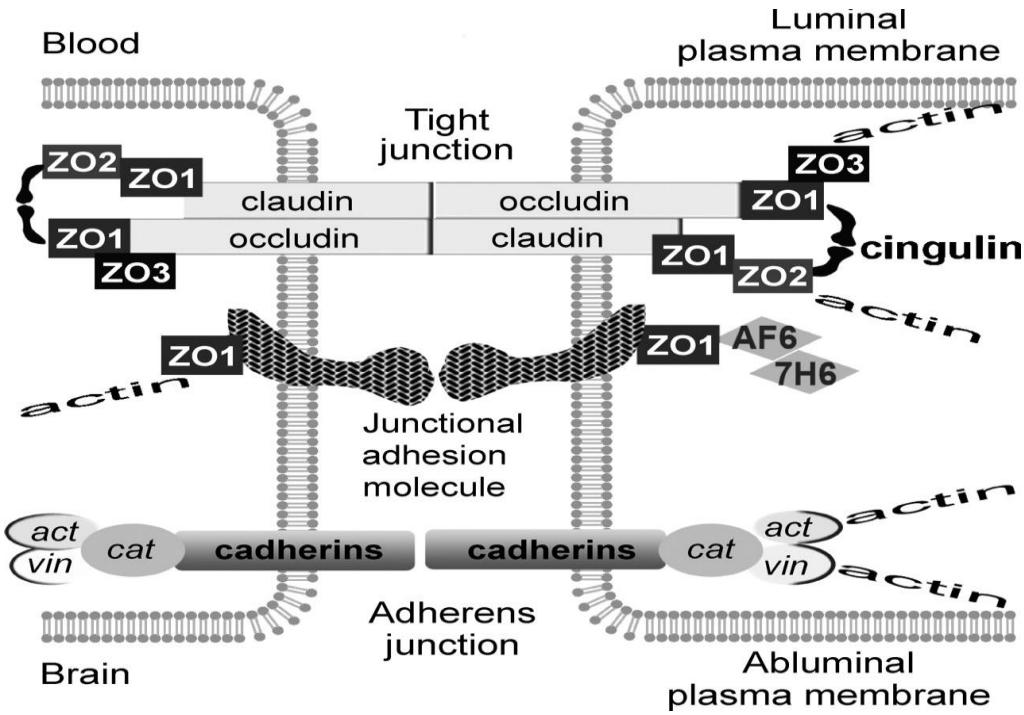

Fig.2. This molecular model of the BBB shows the tight junction proteins found in the BBB, occludin and claudin. These tight junction proteins keep unwanted molecules out of the brain. Testing to see if occludin and claudin are over-expressed or under-expressed in the diabetic hypercholesterolemia pigs may help to show how exactly these conditions act as a precursor to AD.

4.3. CELL PROFILER PROCESSING

The DMHC pigs used in this study have altered expression levels of claudin V protein in the BBB. After performing immunohistochemistry on the pig brain slides, a count was done on how many positive stained vessels there were for claudin V. Figures 3 and 4. indicate how Cell Profiler processes the slides before beginning the vessels count. Figure 4 shows all of the positive stained vessels which expressed claudin V. Below the colorful image is the exact count of positive stained vessels expressing claudin V.

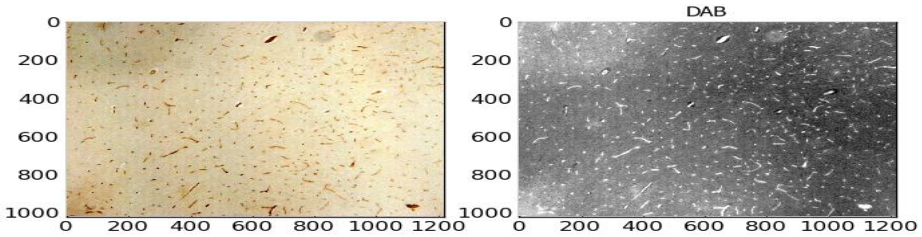

Fig.3. On the left is the original image of a section of brain tissue immunostained to reveal claudin V-positive blood vessels. A threshold is set so that very lightly stained vessels are not counted. The picture to the right shows the claudin V positively stained vessels that were darkly stained enough to be counted by cell profiler.

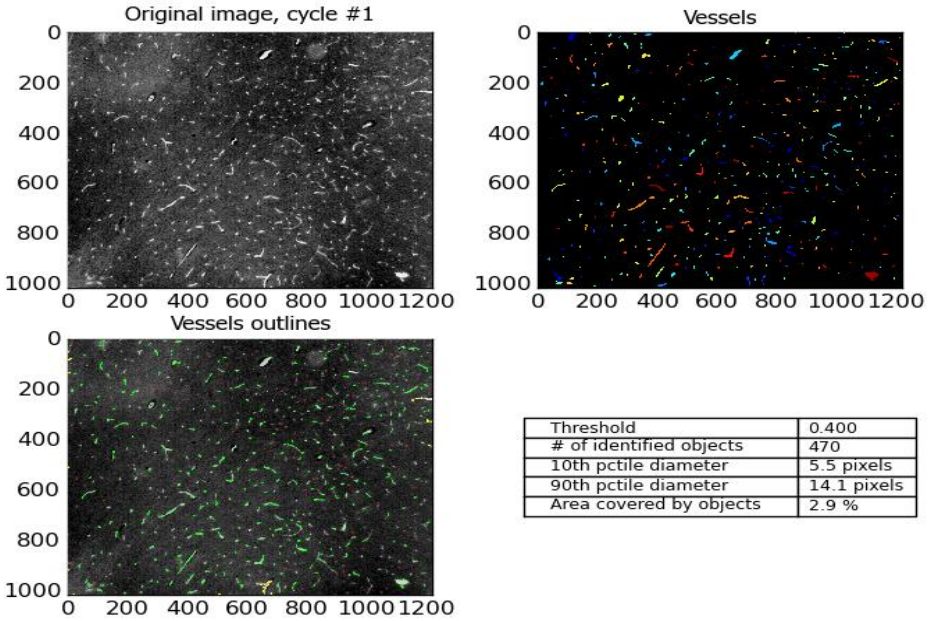

Fig.4. Cell profiler is able to count all of the stained claudin V-positive blood vessels. A threshold is set in order to make sure that only claudin V stained blood vessels are found. Images are first converted to black and white, which also discards any vessels that are below the preset threshold. The white lines are counted as the positive stained claudin V blood vessels. For this study cell profiler was told only to count how many vessels are stained positive and the fraction of the total area covered by the vessels.

4.4 Expression Levels of Claudin and Occludin

Figures 5, 6 and 7 are graphs that show the average count of claudin V-positive vessels in each brain region of the pig. As shown, each brain region demonstrates deviated expression levels of claudin V compared to the normal control pigs. Claudin V expression is greater in the DMHC pigs compared to the control in all three brain regions. This would indicate that the condition of DM and HC alters the expression level of the tight junction protein, claudin V. The overexpression of claudin V in each brain region is shown to be around the same. This indicates that overexpression of claudin V in DMHC pigs is ubiquitous throughout the pig brain. An increase in claudin V expression level may indicate that claudin V is overexpressed in order to compensate for a breakdown in the BBB; however, further work will need to be performed in order to determine if this is the case. Claudin V expression in drug-treated pigs is also increased compared to control pigs. Darapladib is believed to help in the repair of leaks in the BBB.

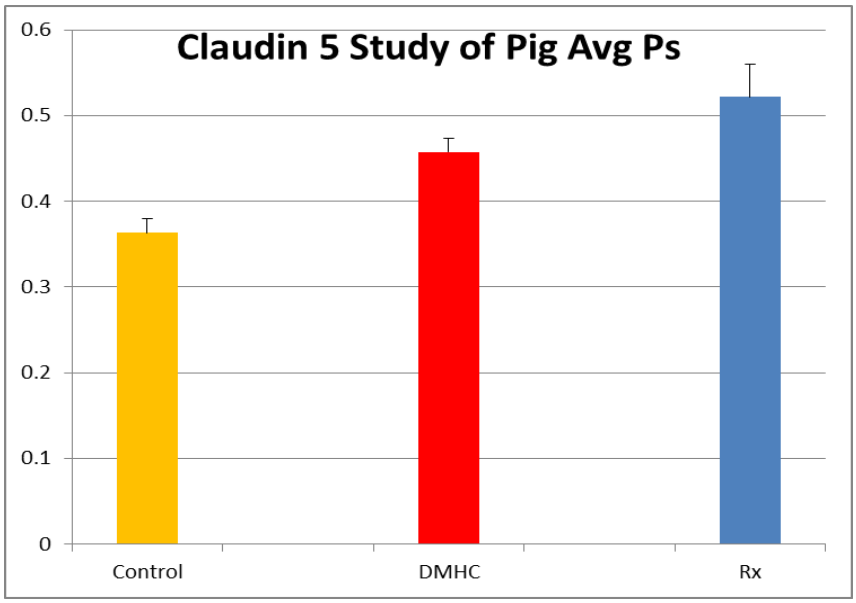

Fig.5. Graph showing the expression levels of claudin V in the posterior brain region (Ps) of control, DMHC, and Rx pig brains. Each group had 250 different images respectively that were used in determining this average count. The Y axis represents number of claudin V positive vessels per unit area.

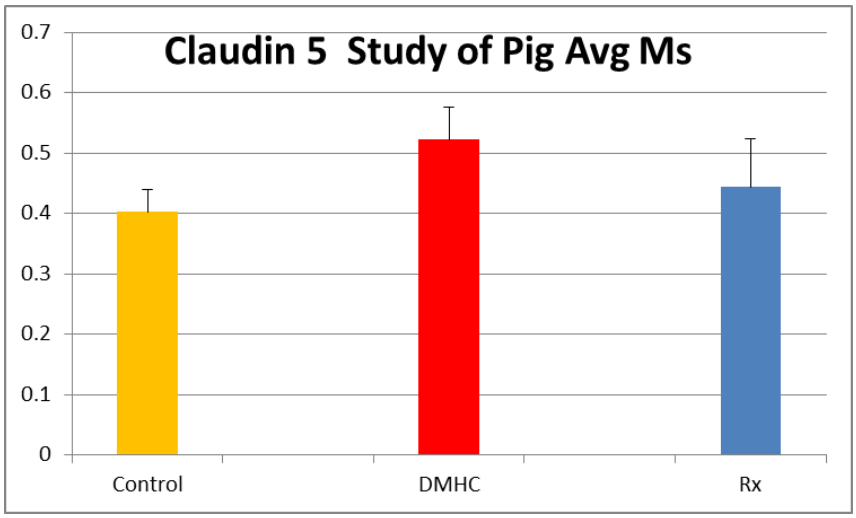

Fig.6. Graph showing the expression levels of claudin V in the middle-brain region (Ms) of control, DMHC, and Rx pig brains. Each subject groups had 250 different images respectively that were used in determining this average count. The Y axis represents number of claudin V positive vessels per unit area.

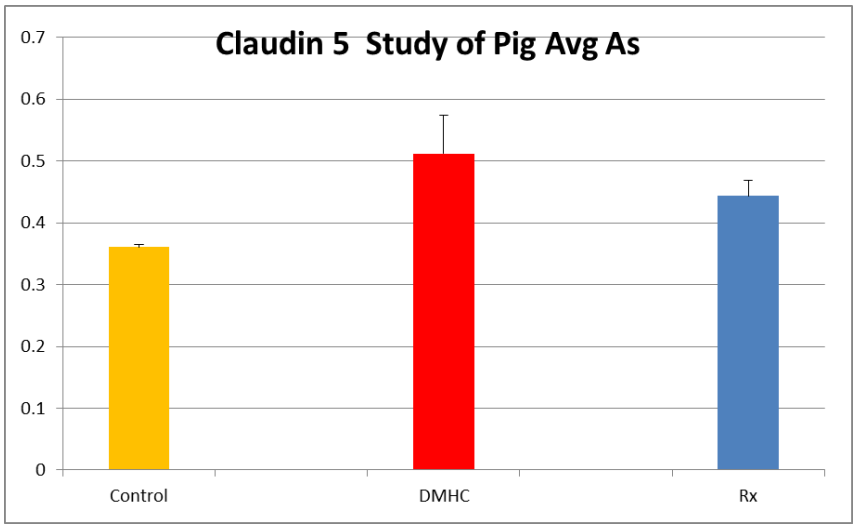

Fig.7. Graph showing the expression levels of claudin V in the anterior brain region (As) of control, DMHC, and Rx pig brains. Each of the subject groups had 250 different images respectively that were used in determining this average count. The Y axis represents number of claudin V positive vessels per unit area.

Occludin did not show the same expression pattern throughout the brain as claudin V. In figures 8, 9 and 10 occludin expression was not always greater in the DMHC pigs compared to control. However, occludin expression is altered in each brain region of the DMHC pig. This confirms that DMHC alters the expression of occludin in the pig brain. Darapladib treated pigs also show a change in expression throughout the brain regions. Like the DMHC pigs, it is not consistent throughout the brain regions.

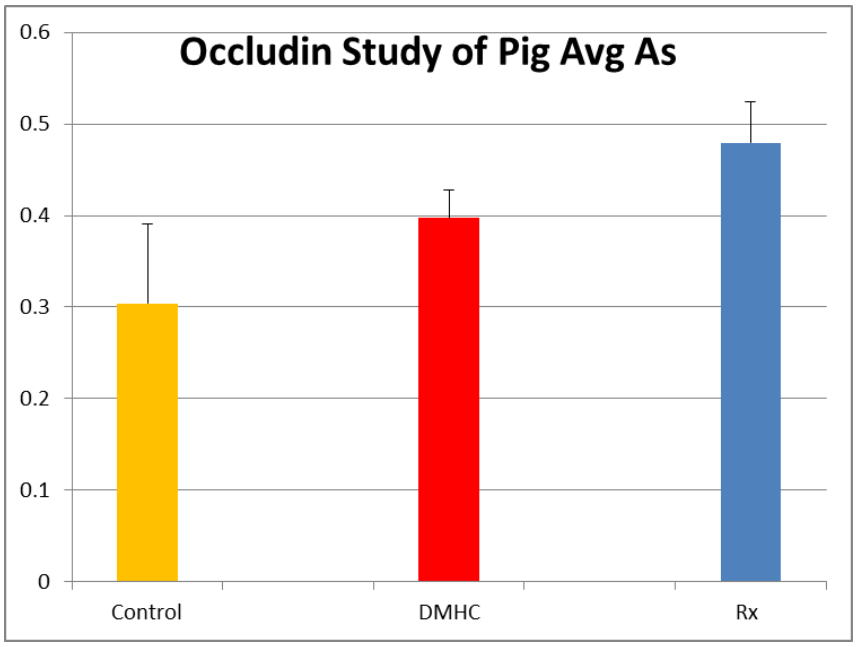

Fig.8. Graph showing the expression levels of occludin in the control, DMHC, and Rx pig brain slices in the anterior region (As). All three sections of the graph had 250 different images respectively that were used in performing this average count. The Y axis represents number of occludin positive vessels per unit area.

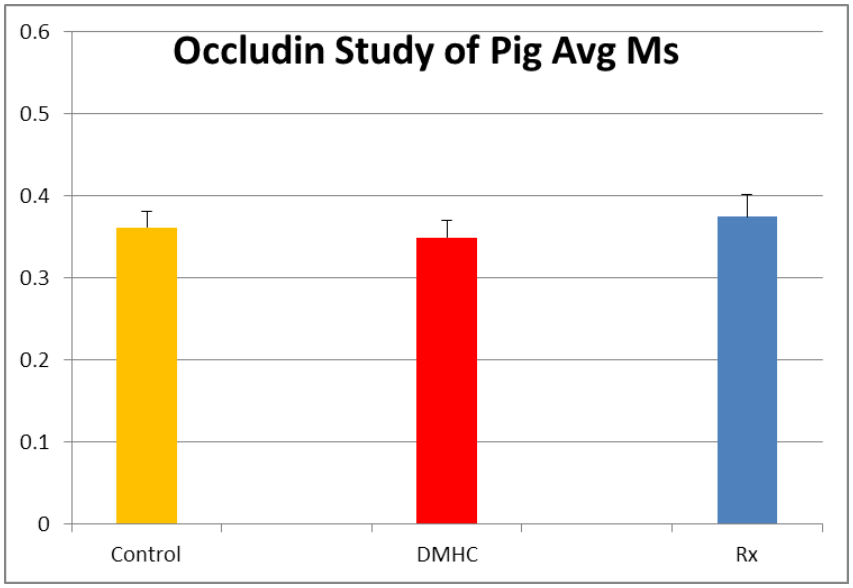

Fig.9. Graph showing the expression levels of occludin in the control, DMHC, and Rx pig brain slices in the middle region (Ms). Values for each group were determined from 250 different images that were used in performing this average count. The Y axis represents number of occludin positive vessels per unit area.

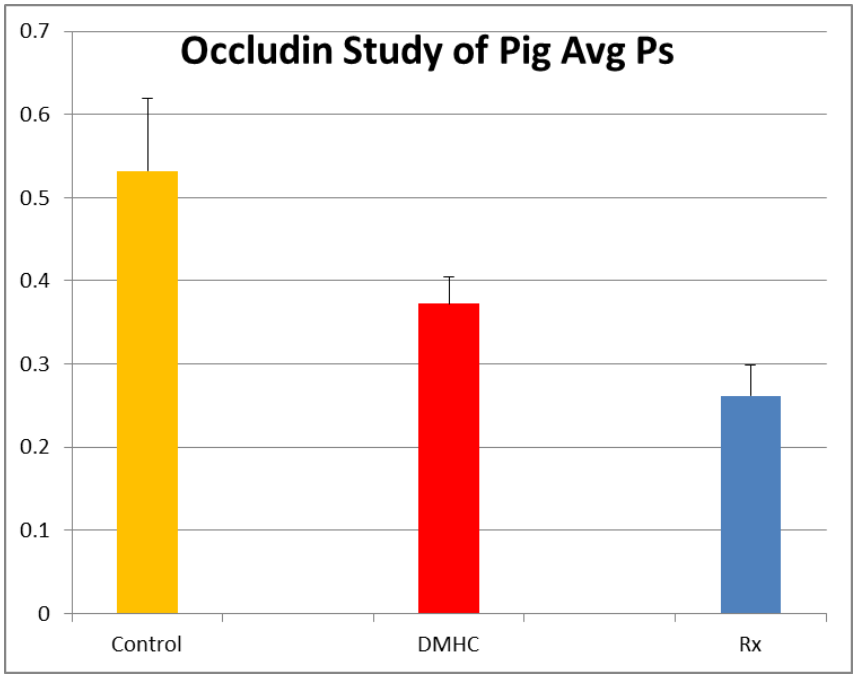

Fig.10. Graph showing the expression levels of occludin in the control, DMHC, and Rx pig brains from the posterior region (Ps). Each value represents 250 different images respectively that were used in determining this average count. The Y axis represents number of occludin positive vessels per unit area.

Chapter 5

Discussion

5.1 Previous Studies Have Shown High Prevalence of Diabetes Mellitus and hypercholesterolemia in AD patients.

It is now recognized that there is a strong correlation between DM, HC, and AD. Many studies have shown this to be true. (Allen et al., 2004; Awad et al., 2004; Carlsson, 2010) A study performed over a five year period followed 1786 patients with mild to moderate AD. The mean age of the patient group was 71 years old. All of the standard procedures were performed to confirm that these patients had AD. 61.9% of the patients had one of the many vascular risk factors. Diabetes mellitus was one of the most prevalent vascular risk factors, accounting for 22.3% of the patients. 13.8% of the patients had hypercholesterolemia. (Park, et al., 2011) Another study was performed by Medicare in the United States on AD patients who were 65 and over. Medicare studied 20% of their clients who had AD and found that 29% of them had diabetes (Alzheimer's, 2012). These vascular risk factors appear to increase a person's risk for AD.

5.2 Diabetes and Hypercholesterolemia May Cause Breakdown of BBB

The fact that such a large percentage of AD patients have diabetes mellitus or hypercholesterolemia shows that these two vascular risk factors are also risk factors for AD, but why they are this is not yet known. The above experiments show that

tight junction proteins namely, claudin V and occludin, demonstrate changes in expression due to these conditions. DM and HC may cause a breakdown of the BBB, which is why changes in claudin V and occludin expression levels were seen. These tight junction proteins form a tight bond at the level of intercellular junctions, keeping the BBB sealed to prevent unwanted molecules in the blood from entering into the brain parenchyma. When leaks form in the BBB, unwanted proteins like autoantibodies enter the brain tissue from the blood and then these proteins may begin to destroy neurons, which could cause AD. Studies have shown that claudin V and occludin are important in sealing the BBB and preventing the disruption of these cell membrane tight junctions that stops BBB leaks. (Matter & Balda 2003)

An experimental study using rats tested the effects of hypercholesterolemia (HC) on the BBB. Rats were fed a 5% cholesterol rich diet over the course of five months. At the end of 5 months, these rats had "spatial memory deficits (meaning a dysfunctional cholinergic system), blood–brain barrier leakage, and inflammation" (Ehrlich & Humpel, 2012). These results indicate that hypercholesterolemia does indeed affect the integrity of the BBB (Ehrlich & Humpel, 2012)

Two similar studies tested the integrity of the BBB, first in human patients who had diabetes and second in rats with induced diabetes human and rats together in study no way. In the human study, 10 control patients and 10 patients with well controlled diabetes were observed. All 20 patients were injected with Gd-DTPA intravenously and MRI imaging was used to examine the brains to see BBB permeability. The diabetic patients showed an increased BBB permeability compared

to the control patients (Starr, et al., 2003). In the second study, rats with induced diabetes also demonstrated regional increases in BBB permeability in the midbrain. Albumin, inulin, and sucrose showed an increased permeability across the BBB in the diabetic rat brains verses the control rat brains (Huber, et al., 2006). Although both of these were preliminary studies, the results reveal that diabetes appears to increase BBB permeability.

DM and HC do not immediately begin to affect the BBB; instead they begin to cause disruptions of the BBB after a long period of time (Huber, et al., 2006). Hence, it is apparent that these two vascular risk factors from DM and HC affect the BBB in some way. We have shown in this porcine study that DM and HC affect the expression levels of both occludin and claudin V, two important proteins in forming tight junctions in the BBB.

5.3 Effects of Glucose Toxicity on Blood Brain Barrier

Previous studies have shown that high glucose levels increase the number of superoxide anions in the body. (Christ et al., 2002) The superoxide anions appear to initially reduce the number of tight junction proteins in the BBB. One study demonstrated a reduction in occludin and claudin V tight junction proteins due to an increase of the superoxide anions, which were caused by high glucose levels. Furthermore, "activation of AMPK helped to maintain the integrity of the blood–brain barrier by suppression of NAD(P)H oxidase and reactive oxygen species production".(Liu et al., 2012) Reduction of the superoxide anions reduced the loss of

claudin V and occludin. This was observed using real time dynamics of claudin V and occludin. The high glucose increased the level of superoxide anions and subsequently caused a decrease in tight junction proteins. However, superoxide anions do not reduce the tight junction proteins indefinitely. (Liu et al., 2012) Eventually, tight junction proteins will increase. This maybe a compensatory mechanism to reduce the increased permeability of the BBB and return to normal physiological conditions.

5.4 Effects of Superoxide Anions on Tight Junction Proteins

Claudin V and occludin are important tight junction proteins that are involved in maintaining the integrity of the blood brain barrier. This study among others has found changes in both claudin V and occludin levels in AD as well as in diabetes mellitus. "Several cytoplasmic signaling molecules, such as Rho, PI3 kinase, protein kinase C (PKC), Ca2+, heterotrimeric G proteins, cyclic adenosine monophosphate (cAMP), and phospholipase C have been localized to these tight junction (TJ) complexes and they may regulate their assembly and disassembly."(Schreibelt et al., 2007) For example, reactive oxygen species selectively activate PI3 kinase and protein kinase B signaling cascades via RhoA. This action may cause a rearrangement of the actin cytoskeleton as well as a spatial redistribution and disappearance of occludin and claudin 5, which would alter BBB integrity. (Schreibelt et al., 2007)

5.5 Hypercholesterolemia Causes Ischemia in Cerebral Microvasculature

Multiple studies have shown that HC increases the amount of AB-42 accumulation in patients with AD. (Refolo et al., 2000; Eckert et al., 2003) However,

little research has been done on how HC affects the BBB. HC has been associated with atherosclerotic plaques. Excess cholesterol leads to high levels of low-density lipoprotein (LDL), which can enter arteries and subsequently form plaques. These plaques can narrow or completely occlude arteries and arterioles. This can result in a lack of blood and oxygen to a particular area. If this occurs the capillaries forming the BBB will receive less blood and oxygen, which may cause dysfunction. The results of this study indicate that high levels of cholesterol cause changes in the expression of tight junction proteins in the blood brain barrier. In Bowman's (2012) study high levels of cholesterol cause impairment of the BBB. It is plausible that HC leads to plaque formation resulting in ischemia to particular capillaries and subsequent damage to tight junction proteins. Chen showed a reduction in tight junction proteins and subsequent leaks in the BBB following ischemic injury. The study was done using fetal sheep and it examined tight junction protein levels at 4, 24, and 48 hours. The levels of tight junctions were lowest 4 hours following the ischemia. However, the levels increased after 24 and 48 hours. This indicates that lack of oxygen will initially cause down regulation of tight junction proteins. (Chen et al., 2012) This study shows increased levels of tight junction proteins, which again may be indicative of a compensatory mechanism following loss of tight junction proteins and subsequent increased permeability.

5.6 Benefits of understanding how diabetes mellitus and hypercholesterolemia affect the blood brain barrier and increase a person's risk of developing AD.

DM and HC appear to increase a person's risk factor for developing AD. Preventing these two diseases through diet, exercise, and a healthy lifestyle should decrease a person's risk of developing AD. However, understanding why these two diseases are risk factors may help in the development of new drugs and therapies that may prevent this pathway, which causes AD. If claudin V and occludin are involved in this pathophysiology, which is suggested by the results listed above, targeting these two proteins in further studies may provide the answers needed to move forward.

Many preliminary studies have been done on how vascular risk factors such as hypercholesterolemia and diabetes mellitus influence the integrity of the BBB. It is important that more in depth studies begin to explore why exactly these two diseases affect the BBB. These two diseases are prevalent in patients with AD and finding out why they appear to be risk factors for AD will open up a myriad of therapies to prevent patients with these diseases from getting AD.

Chapter 6

Summary and conclusion:

The increase in both claudin V and occludin in DMHC pigs indicates that the BBB is affected by these vascular diseases. The increase shown in the data is likely due to a compensatory mechanism following leaks in the vasculature of the BBB. It is clear that claudinV and occludin are involved in sealing the blood brain barrier and when they are altered leaks occur. Other studies mentioned above show the initial decrease in tight junction proteins and a subsequent increase days following. It is probable that the production of these proteins increases following leaks in the BBB.

More animal studies must be completed to observe what happens to tight junction proteins immediately following BBB leaks, and then some time following the leaks. Long term studies must continue to monitor pig brains affected by DMHC in order to check tight junction protein levels at various stages of the diseases. Tight junction protein levels must be tested at particular intervals following glucose toxicity and high cholesterol diets. This may be able to indicate how much the tight junction proteins can withstand before they begin to leak and reduce. It may also reveal at what point the tight junction proteins begin to increase and why.

Currently there is no cure, prevention, or early diagnostics for AD. There is much to be done in the field of AD research. Much hope lies in the hands of the scientists who are currently testing and studying the causes, treatments, and diagnostics for this disease. One of the most important aspects is finding out how

leaks form in the BBB. There are many different theories as to why the BBB forms leaks, and until the cause is found scientist cannot begin to repair it. This disease is likely to inflict many people and the need for answers will continue to grow. Once scientists are able to find the actual cause and pathway of the disease the therapies and diagnostics will become much easier.

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Attributes

Abbreviations

AD	Alzheimer’s disease
DMHC	Diabetes mellitus hypercholesterolemia
DM	Diabetes mellitus
HC	Hypercholesterolemia
BBB	Blood Brain Barrier
a7nAChRs	alpha 7 nicotinic acetylcholine receptors
LDL	Low density lipoprotein