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Insights into Alzheimer's Disease: The Levels of Signaling Proteins in Brain of Control Subjects Versus Brain from Subjects with Mild Cognitive Impairment

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I am a Senior working on a B.S. in Biology. I am Executive Vice-President of the National Society of Collegiate Scholars (2008-2009) and an AMSTEMM Research Fellow. Also, I have received a eUreKa! Research Grant

My future plans are to attend medical school. This submission relates closely to my future plans because I am highly interested in neuroscience, gerontology, and how Alzheimer's disease (AD) significantly affects the character of the person who is diagnosed with it. I am intrigued by this disease's power to radically

change a person's life. Not only does Alzheimer's disease cause severe memory loss, this disease also causes the ruthless diminishment of cognitive thinking skills. Some people who have late-onset AD (LAD) lose the ability to perform simple, daily tasks normal people take for granted, such as the ability to speak or to remember one's own name.

By working with Dr. Butterfield, I have gained much insight and knowledge regarding AD. I was privileged to work with Dr. Butterfield's research group, considering that I had no prior research laboratory experience. Dr. Butterfield mentored me through this research of AD for over a year, and he has given me great advice on not only school-related subjects, but also on life in general. He is someone to whom I can go for advice on applications to graduate school, on study questions in biology, chemistry, and biochemistry, and on what I should do to make myself a stronger student inside and outside the classroom. I am honored that someone as prestigious as Dr. Butterfield was able to give me a hands-on experience in science. In addition, I have gained many friends. All of the graduate students, post-doctorates, and research associates always offer a lending hand, and no is ever excluded.

My most important extracurricular activities are volunteering at St. Joseph Hospital, summer clinical experiences with physicians from Harlan Appalachian Regional Healthcare Hospital, playing the piano, exercising (which is extremely important in the prevention of Alzheimer's disease), cooking, and baking.

This work has been presented at the Kentucky Academy of Science, the AMSTEMM Research Colloquium, the National Conference on Undergraduate Research (NCUR), and the UK Showcase of Undergraduate Scholars. This research work will be combined with the work of Dr. Sultana and plans for submission for publication in a professional journal are underway.

As a research student on AD, I am quite optimistic that someday AD will be a thing of the past. This success can and will be achieved as research advances and new unknowns are solved. I believe that researchers will be capable of solving all of the unknown facets of this disease and will be able to synthesize a definite cure one day. That is my hope, and I want to be a part of the solution. Ultimately, I wish that everyone can attain a full, satisfying life — a life filled with memories unforgotten.

Insights into Alzheimer's Disease: The Levels of Signaling Proteins in Brain of Control Subjects Versus Brain from Subjects with Mild Cognitive Impairment

eUreKa! Research and Creativity Program Project

**Faculty Mentor:
Professor D. Allan Butterfield,
Department of Chemistry**

The study completed by Ms. Tiu is outstanding. It is rare that an undergraduate is allowed to use human brain specimens, but Ms. Tiu is so careful in her work that an exception was made for her. Ms. Tiu found levels of key brain proteins are altered in the brains of subjects with mild cognitive impairment (MCI), arguably the earliest detectable form of Alzheimer's disease (AD). Our laboratory's previous research had shown that oxidative damage to the brain in MCI is present. The proteins whose levels are altered in MCI likely reflect this oxidative damage, and these proteins are involved in signaling and/or neuroinflammation. Consequently,

the significance of this research is that the altered levels of these proteins determined by Ms. Tiu in the brains of subjects with MCI strongly support the notion that oxidative stress-induced brain protein alterations are important in the progression of AD. Ms. Tiu conducted this research and wrote the paper by herself, with some editing assistance from me.



Abstract

The purpose of this study was to measure the expression levels of key signaling proteins in brain tissue from subjects with mild cognitive impairment (MCI) compared to control subjects. MCI is considered to be the beginning phase of Alzheimer's disease (AD). Tumor necrosis factor alpha (TNF- α), nuclear factor kappa beta (NF- κ β), phospho Bad (pBad), and ubiquitin C-terminal hydrolase-L1 (UCH-L1) are four of the proteins that were investigated. Trends of either decreases or increases in protein expression levels in MCI vs. control brain were investigated. Western blot analysis was used in order to identify these trends. These signaling protein levels are reportedly up-regulated or down-regulated in the AD brain; consequently, investigating protein expression levels in MCI brain may provide insight into how these proteins contribute to the transition from MCI to AD.

Introduction

Alzheimer's disease (AD) is a disheartening illness to not only those who have developed it, but also to family and friends of AD patients. According to current research, it is estimated that 14-16 million Americans and more than 22 million people around the world are expected to develop AD in the near future, unless an intervention can be developed (Butterfield et al., 2007). However, that much-anticipated intervention is closer to reality due to the efforts of Professor Butterfield and his team.

Mild Cognitive Impairment (MCI) is considered to be the "intermediate stage between normal aging and the AD brain" (Butterfield et al., 2007). In the early stages of MCI, many people go on living their daily lives quite normally. However, in amnesic MCI there is a noticeable decline of memory compared to prior years, but there are no signs of dementia in the early stages. Still, some researchers consider MCI to be the earliest stage of AD. Using redox proteomics to investigate molecular aspects of AD and MCI, Professor Butterfield's team has tracked and recorded the degrading effects of oxidative stress (Butterfield et al., 2007).

Earlier studies have illustrated the importance of oxidative stress in the pathogenesis of AD. Oxidative stress is caused by an imbalance between the production of toxic reactive oxygen species (ROS) and the body's antioxidant systems. Protein oxidation can lead to extracellular amyloid plaques, intracellular neurofibrillary tangles, oligomers of amyloid β -peptide (A β), and synapse loss (Butterfield et al., 2007). The A β (1-42) peptide is the peptide of interest in the study of AD pathology. A β (1-42) is a peptide that is derived from the proteolytic cleavage of a Type I integral membrane protein known as amyloid precursor protein (APP) and is thought to underlie

the pathogenesis of AD (Butterfield et al., 2007).

Protein oxidation causes notable adverse effects. Oxidation of proteins can cause protein dimerization, in which two molecules of the same chemical composition can form a condensation product. In addition, protein oxidation can lead to denaturation of proteins, a process in which a protein unfolds from its usual structure, and its normal conformation is lost. This process is detrimental because the unfolding allows the hydrophobic ("water-fearing") amino acid residues to be exposed to the aqueous environment. This exposure can lead to a loss of structural or functional activity and cause protein aggregation, such as tau aggregation that is evident in the AD brain.

Tau aggregation is responsible for forming the neurofibrillary tangles, while senile plaques are composed of extracellular, fibrillar A β (1-42) that histopathologically characterize the AD brain. The senile plaques are associated with activated astrocytes and copious amounts of microglia at the site of deposition of A β (1-42) in AD (Bernhardi and Eugenin, 2004). Furthermore, the activation of the inflammatory response is thought to be crucial in quantifying the rate of the neurodegenerative process (McGeer and McGeer, 1998). β -Amyloid activates glia to synthesize an assortment of inflammatory factors such as tumor necrosis factor-alpha (TNF- α), reactive oxygen species (ROS), nitric oxide (NO) and inducible nitric oxide synthase (iNOS), which may increase the rate of the development of AD (Jiang et al., 2007).

Materials

All chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of nitrocellulose membranes (Bio-Rad, Hercules, CA), electrophoretic transfer system (Trans-blot Semi-dry Transfer Cell; Bio-Rad), and Western blotting (Calbiochem, LA Jolla, CA).

Subjects

The University of Kentucky Rapid Autopsy Program of the Alzheimer's Disease Clinical Center (UK ADC) provided frozen hippocampus samples from MCI subjects and age-matched controls. The patients were diagnosed with probable MCI according to criteria produced by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association. The control subjects did not have any neurological disorders nor did they have a history of dementia. They had undergone annual mental status testing and semiannual physical and neurological examinations as part of the UK ADC normal volunteer longitudinal aging study. Table 1 describes the background of the patients.

Table 1. Characteristics of control and MCI patients (mean ± SD).

Demographic variables	Control Subjects	MCI Subjects ¹
Number of subjects	7	7
Gender (male/female)	3/4	3/4
Postmortem Interval (hours)	2.87 ± 1.14	3.125 ± 1.033
Brain weight (grams)	1260 ± 120	1120 ± 61
Braak ² stage	I-II	III-V

1. MCI: mild cognitive impairment
2. The standard, six-stage description of a patient's level of AD, from extremely mild to full-blown dementia, named for the German researcher who developed the scale.

Sample preparation

The brain tissues (hippocampus) from controls and MCI subjects were homogenized in ice-cold isolation buffer containing 10mM HEPES buffer, 137mM NaCl, 4.6 mM KCl, 1.1 mM KH₂PO₄, and 0.6 mM MgSO₄, as well as proteinase inhibitors leupeptin (0.5 mg/ml) and pepstatin (0.7 mg/ml). Homogenates were centrifuged at 14,000 g for 10 min to remove debris. The supernatant was extracted to determine the total protein concentration by the BCA method.

Twenty µl of either 150 µg or 100 µg of control and MCI samples from the hippocampus (one of the first regions of the brain to suffer damage from AD) were used in the 1D Western blots. 1D Western Blots were used to determine the protein expression levels in control and MCI brain tissues. Gel electrophoresis was utilized to separate the denatured control and MCI brain proteins, and the proteins were then transferred to a nitrocellulose membrane. Primary and secondary antibodies detected the proteins that were on the blot.

The science underlying tagging antibodies is actually quite simple. First, an animal such as a rabbit is injected with a protein, such as TNF-α. The rabbit's immune system then produces antibodies against the foreign protein, TNF-α. The antibodies are then collected and are called primary antibodies. A second animal, such as a goat, is injected with the primary antibodies that the rabbit's immune system created. The goat's immune system then creates antibodies against the newly injected primary antibodies and the secondary antibodies are thus created.

Western blotting analysis

In the process of Western blotting, a primary antibody is added to the nitrocellulose membrane in order to tag the protein of interest, such as TNF-α. The secondary

antibody then tags and recognizes the primary antibodies. Specific substrates, 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium (BCIP and NBT), are then used to catalyze the reaction and develop the blot so that the proteins can be seen. A purple color is the result of the reaction. A distinctive band of the specific protein is expected to show up first, especially with monoclonal antibodies.

There are two different kinds of antibodies used in this project — monoclonal and polyclonal antibodies. Monoclonal antibodies are specific for certain amino acid sequences and their motifs. A blot that uses monoclonal antibodies usually shows fewer bands than polyclonal because monoclonal antibodies are more specific to the particular protein's amino acid sequence. With the case of polyclonal antibodies, more bands show up because repeating amino acid motifs in proteins other than the particular protein of interest (TNF-α for example) are tagged as well. However, because each protein has its own molecular weight, determining which band belongs to the protein of interest is not difficult. The blots are then scanned with Adobe Photoshop and quantified with Scion Image (PC version of Macintosh-compatible NIH Image) software.

Tumor Necrosis Factor-Alpha, TNF-α

Tumor necrosis factor-alpha (TNF-α) is a member of a cytokine family that can cause cell apoptosis (programmed cell death) and cellular proliferation and differentiation. Cellular differentiation can be observed in macrophages, inflammatory cells that originate from white blood cells. TNF-α is produced by specific cells of the immune system in response to adverse conditions, such as exposure to ionizing radiation, elevated temperature, viral infection, or toxic chemical agents such as those used in cancer chemotherapy (Karp, G., 2005; Tangpong et al., 2006). TNF-α is a pro-inflammatory cytokine that participates in inflammation, cell survival, and cell death processes (Gomez-Chiarri et al., 1994) and can trigger cell death through cell apoptosis and necrosis.

This particular cytokine is a signaling protein that is secreted from immune system cells that ultimately changes the action of other immune cells. TNF-α regulates immune cells and causes inflammatory responses. These inflammatory responses are necessary in order to defend the body against pathogens. However, increased levels of inflammation are evident in neurodegenerative disorders and are implicated in the etiology of Alzheimer's disease, Parkinson's disease, and ischemia (Lovell et al., 1998; Liao et al., 2001; Gao et al., 2002). In mice treated with pro-oxidant adriamycin or in AD brain, neuroinflammation arbitrated from glia overproduction or dysfunctional regulation of TNF-α

is observed (Jiang et al., 2007; Tangpong et al., 2008).

Mechanism

In its normal mechanism of signaling cell apoptosis, TNF- α first binds to its receptor, either TNFR1 or TNFR2. TNFR1 is the receptor that is constitutively expressed in most tissues. The activated receptor then binds TRADD and FADD, two cytoplasmic adaptor proteins, and procaspase-8 to create a complex that is located on the inner surface of the plasma membrane. FADD and TRADD interact with each other in regions known as death domains, and procaspase-8 and FADD interact with each other in what are known as death effector domains. Once assembled together, the procaspase molecules cleave each other to create Caspase-8, an initiator complex that directs the cell to its final destination of cell apoptosis. It is important to note, however, that after TRADD binding, two other pathways can be initiated. They will be discussed later.

Hypothesis and Data

Because neuroinflammation is elevated in the AD brain, I hypothesized that neuroinflammation can be observed in the MCI brain. Therefore, TNF- α levels are hypothesized to be increased in the MCI brain samples. The results thus far confirm my hypothesis. Brain tissue was obtained from five different subjects ($n = 5$) for both control and MCI. There is a clearly significant increase in TNF- α protein levels in MCI. ($p < 0.01$). There is variation due to the biological nature of the samples from human individuals.

Nuclear Factor-Kappa Beta, NF- $\kappa\beta$

As explained above, after TRADD binding, two other pathways other than cell apoptosis can be initiated. One pathway is the activation of NF- $\kappa\beta$. The other pathway is the activation of the MAPK pathways. The protein in this particular study was the p65 subunit of NF- $\kappa\beta$.

Mechanism

In its normal function, after TRADD binds to the death domain, TRADD assembles TRAF2 and RIP, a serine-threonine containing kinase. TRAF2 then recruits protein kinase, IKK, a multi-component protein. This recruitment permits RIP to activate IKK. An inhibitory protein that is bound to NF- $\kappa\beta$, known as I, inhibits NF- $\kappa\beta$ from translocation. I is then phosphorylated by IKK and is degraded. This process releases NF- $\kappa\beta$ so that it can translocate to the nucleus. NF- $\kappa\beta$ is a transcription factor that initiates the synthesis of proteins that are involved in anti-apoptotic factors,

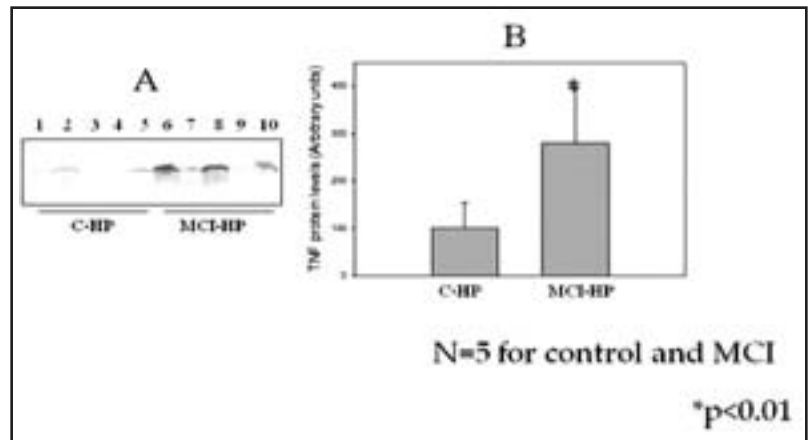


Figure 1. TNF- α Protein Levels in Control and MCI Hippocampus

(A) Western blot with ten lanes. Lanes 1-5 are control samples. Lanes 6-10 are MCI samples. (20 μ l of 100 μ g samples were added to each well.) Lanes 6 and 8 display the significant increase in TNF- α protein levels. (B) Graphical analysis of control and MCI band intensities, respectively. The graph illustrates the significant increase in TNF- α from control and MCI comparison. ($p < 0.01$). C-HP, control hippocampus; MCI-HP, MCI hippocampus.

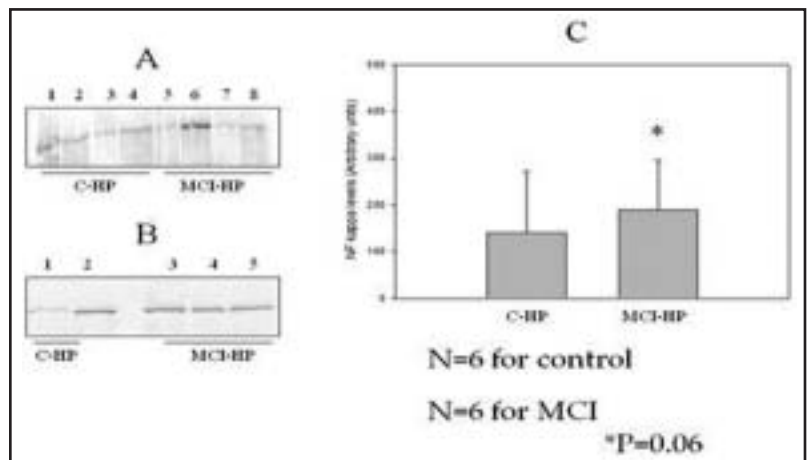


Figure 2. NF- $\kappa\beta$ Protein Levels in Control and MCI Hippocampus

(A) Western blot with eight lanes. Lanes 1-4 are control samples. Lanes 5-8 are MCI samples. (20 μ l of 100 μ g samples were added to each well.) Lane 6 of MCI clearly displays a higher intensity of protein concentration than the control samples and illustrates an increase in NF- $\kappa\beta$ protein levels in MCI. Lane 7's value was thrown out after a Q-test was performed (B) Lane 1 and 2 are control. Lanes 3-5 are MCI samples. (C) Graphical analysis of control and MCI band intensities, respectively. The graph illustrates an increase in NF- $\kappa\beta$ from control and MCI comparison. C-HP, control hippocampus; MCI-HP, MCI hippocampus. ($p = 0.06$; it is near significant)

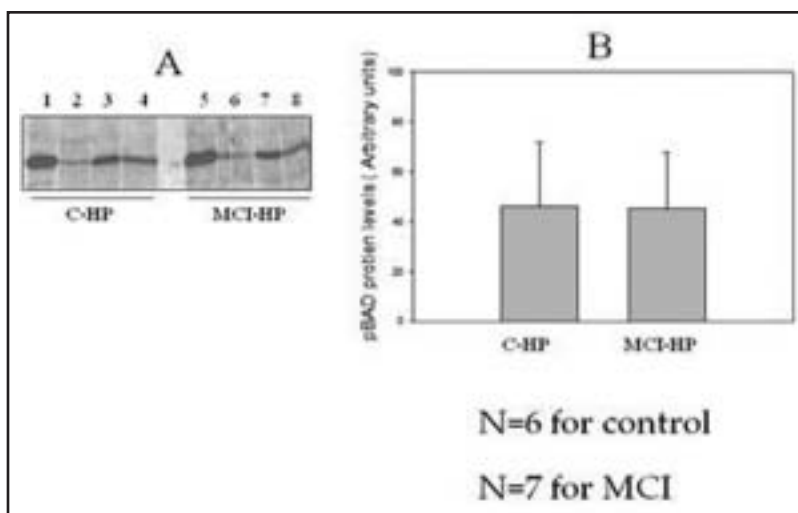


Figure 3. Phospho Bad Protein Levels in Control and MCI Hippocampus (A) Western blot with eight lanes. Lanes 1-4 are control samples. Lanes 5-8 are MCI samples. (20 μ l of 100 μ g samples were added to each well.) (B) Graphical analysis of control and MCI band intensities, respectively. The graph illustrates no change from control and MCI comparison. C-HP, control hippocampus; MCI-HP, MCI hippocampus.

inflammation, and cell survival and proliferation.

Hypothesis and Data

Because p65 of NF- κ B is a transcription factor that mediates the transcription for cell survival and anti-apoptotic factors, I hypothesized that there will be an increase in p65 of NF- κ B in the MCI brain, because neuroinflammation and pro-apoptotic factors are expected to be increased in the AD brain. NF- κ B can provide the brain with neuroprotection, and if NF- κ B is not released into the nucleus, the result will be a lack of important, protective proteins. According to prior research, p50/p65 of NF- κ B, has been reported to be activated in AD brain (Kaltschmidt et al., 1997; Yan et al., 1995; Yoshiyama et al., 2001). According to that research, p65 is expected to be increased in AD brain. However, is there an increase of p65 in MCI brain? There have been several studies that demonstrate that, if NF- κ B is introduced to an inhibitor, or even its inhibitory protein, I κ B, is blocked with a repressor, the protective effects are nullified.

Nakao et al. (2008) demonstrated that galantamine (a drug that is currently being used to treat mild to moderate AD) and its protective effects are nullified with the inhibition of NF- κ B or the repression of its bound protein, I κ B. This finding suggests that NF- κ B is crucial in the reduction of inflammation and the mechanism of defense. Therefore, it is hypothesized that there will be an increase in NF- κ B levels in MCI. My results support

the hypothesis of a trend toward an increase in NF- κ B levels in MCI brain, and the results also illustrate near significance as assessed by the Wilcoxon p-value of 0.06.

Phospho Bad (Serine 112) (Phosphorylated Bcl-2 associated death promoter)

Bad is a member of the B cell leukemia-2 (Bcl-2) gene family. When Bad is phosphorylated, it is called phospho Bad (p-Bad). Serine 112 phospho Bad was used in this study. Alterations in phospho Bad antagonize apoptosis function in Bad (Jeong et al., 2007). Oxidative stress can cause alterations in protein expression and perhaps efficiency. Bad's main role is to signal cell apoptosis to cells that may be damaged, from oxidative stress for example. Phosphorylation initiates Bad to signal pro-apoptotic pathways in order to rid the brain of damaged components of a cell.

Mechanism

Oxidative stress is one factor that causes cellular stress that ultimately leads to cell apoptosis. Cellular stress causes a pro-apoptotic factor such as Bad to insert itself into the outer mitochondrial membrane. The insertion of the protein, Bad, then signals the release of cytochrome c molecules from the intermembrane space of the mitochondria. When the cytochrome c molecules are translocated to the cytosol, they form a multi-subunit complex with caspases that then, in turn, activate the downstream executioner caspases that cause apoptosis.

Hypothesis and Data

Because phospho Bad's function is to cause cell apoptosis, it is hypothesized that there will be a decrease in phospho Bad protein levels in MCI brains in order to provide neuroprotection from oxidative stress. The reason for this decrease is that when an environmental factor, such as oxidative stress, affects the brain cells, phospho Bad is signaled to cause pro-apoptotic caspases to rid the brain of damaged cells and to regulate the cell death processes. In the normal brain, I hypothesized that a decrease in phospho Bad activity or a loss of function could result in the aggregation of unwanted proteins.

The data below illustrate no change in phospho Bad protein levels in MCI brain, suggesting that these unchanging levels contribute to unwanted protein accumulation and neuronal toxicity in AD brain. Yoon et al. (2006), support these data, stating that the "cultured neurons have shown that okadaic acid (OA) evokes tau phosphorylation to initiate a neurodegeneration that resembles the pathogenesis of AD. Western blotting revealed that ... expression of Bad was decreased as early as 4 hours after OA treatment, although the level of phospho Bad was relatively constant." These

levels were observed in cultured cortical neurons.

Ubiquitin Carboxyl Terminal Hydrolase L1 (UCH-L1)

Ubiquitin C-terminal hydrolase L1 (UCH-L1) is an enzyme that is a main component of the ubiquitin proteasome system and has catalytic hydrolase properties that can hydrolyze peptide-ubiquitin bonds and recycle ubiquitin monomers to be used again in the same process (Healy et al., 2004). UCH-L1 is a member of the deubiquitylating enzymes and is one of the most abundant proteins in the brain. Whereas other UCH members are ubiquitously expressed, UCH-L1 is selectively expressed in neurons (Sakurai et al., 2006; Wilkinson et al., 1989). Moreover, it is an enzyme involved in the process of proteolytic degradation of misfolded or damaged proteins by the proteasome (Castegna et al., 2002).

Analysis of proteasome function in post-mortem human AD brains has shown a strong inhibition of activity in the proteasome and lower protein levels for UCH-L1, suggesting that the neuronal ubiquitin-proteasomal system is linked to the pathogenesis of AD (Gong et al., 2006). Research from our lab also confirmed a decrease of UCH-L1 activity in AD brain. According to Sultana et al. (2006), the measurement of enzymatic activity of CA II, UCHL-1, and enolase from AD hippocampus revealed decreased activity compared to control.

UCH-L1 is important for proteasomal-mediated clearance from the brain of aggregated or misfolded proteins. Protein aggregation, such as tau aggregation, is seen in the AD brain. The decline of UCH-L1 proteasomal activity likely contributes to protein aggregation and thus neuronal toxicity. Less UCH-L1 in MCI brain is predicted to result in more ubiquitinated proteins, decreased activity of the 26S proteasome, and consequently, an accumulation of aggregated and damaged proteins. All were observed in MCI brain.

Because there is evidence of a decline in UCH-L1 activity in AD brain (Sultana et al., 2006), I hypothesized that there will be a decline of UCH-L1 protein levels in the MCI brain as well. The results below support my hypothesis.

Statistics

For all trials, a Student's t-Test was performed in order to determine significance. Q-test analysis was also performed to determine the outliers for statistical analysis. The results are presented as the mean plus or minus the standard deviation. Because biological samples were used in this project, variation was expected to occur.

Discussion

Because NF- κ B is downstream from TNF- α , it is hypothesized that there will be a proportional relationship between the two proteins. If TNF- α increases in the

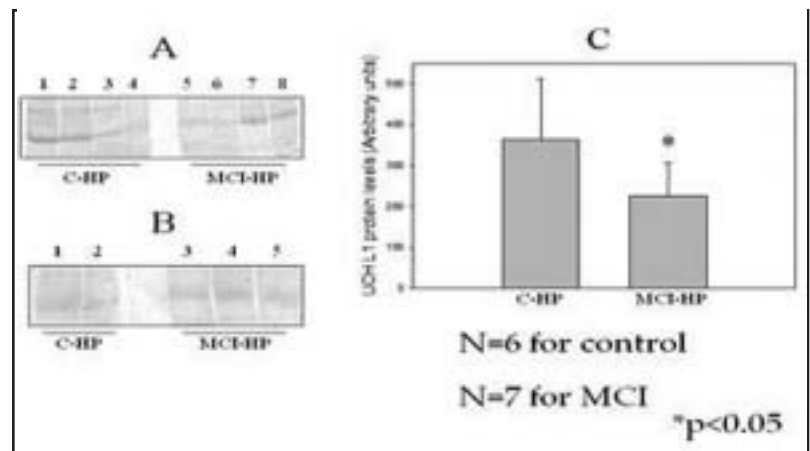


Figure 4. UCH-L1 Protein Levels in Control and MCI Hippocampus (A) Western blot with eight lanes. Lanes 1-4 are control samples. Lanes 5-8 are MCI samples. (20 μ l of 100 μ g samples were added to each well.) Lanes 1 and 2 (control) clearly display higher intensities than the MCI samples and illustrate the significant decrease in UCH-L1 protein levels in MCI. (B) Lanes 1 and 2 are control samples. Lanes 3-5 are MCI samples. (C) Graphical analysis of control and MCI band intensities, respectively. The graph illustrates the significant decrease in UCH-L1 from control and MCI comparison. ($p < 0.05$). C-HP, control hippocampus; MCI-HP, MCI hippocampus.

brain, then NF- κ B is expected to increase as well. The increase in oxidative stress creates a signal that anti-apoptotic factors from NF- κ B need to be synthesized.

Phospho Bad is pro-apoptotic, which means that it causes cell death. When TNF- α is activated, because of oxidative stress for example, NF- κ B is activated in order to create proteins that can counteract the oxidative stress. Phospho Bad activates downstream caspases that ultimately lead to cell apoptosis. The unchanging levels of phospho Bad offer an explanation if there is an accumulation of unwanted proteins and neuronal toxicity in MCI.

The unchanging levels of phospho Bad in AD brain can be tied to the decrease in UCH-L1 activity. UCH-L1 is known to be decreased in AD brain (Castegna et al., 2002; Sultana et al., 2006). This finding means that its normal functional levels are decreased and therefore lead to histopathological characteristics that are evident in the AD brain. For example, less UCH-L1 in MCI brain is predicted to result in more ubiquitinated proteins, decreased activity of the 26S proteasome, and, consequently, an accumulation of aggregated and damaged proteins. All are observed in MCI brain.

UCH-L1 is a member of the deubiquitylating family. It is part of the proteasome pathway that

rids the brain of protein aggregations and misfolded proteins. UCH-L1's down-regulated levels that are evident in AD brains contribute to neuronal toxicity, because unwanted proteins accumulate in the brain. Because oxidative stress signals TNF- α production and, therefore, NF- κ B is signaled to produce proteins that help to counteract oxidative stress, phospho Bad is signaled to induce apoptosis to dispose the damaged proteins.

UCH-L1 is affected by oxidative stress as well. Because UCH-L1's proteasomal and degradative properties are decreased in both MCI and AD brain, this means that UCH-L1 cannot rid the brain of damaged, unwanted proteins. This failure will result in protein aggregation and neuronal toxicity.

Conclusion

Through this research, new insights into the histopathology and biochemical alterations observed in MCI brain have been found. Data presented in this paper illustrated that there is an increased level of oxidative stress in MCI supported by increased levels of TNF- α , NF- κ B, and decreased levels of UCH-L1. Phospho Bad's unchanging protein expression levels could mean that there is no activation of pro-apoptotic pathways stimulated by this pathway, and that could lead to an accumulation of damaged components in brain.

Acknowledgements

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