EFFECTS OF IMATINIB METHANESULFONATE ON

INFLAMMATION-INDUCED AMYLOID-BETA

PRODUCTION

by

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TABLE OF CONTENTS

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INTRODUCTION

As far back as records show, there have been countless reports of age-dependent forgetfulness. As early as a few hundred years ago, there are documented descriptions detailing the progression of the elderly slowly exhibiting a mental decline, eventually resulting in a complete loss of memory. The fear of losing one's identity was a constant threat looming over society. Because of this, autopsies were performed, various treatments were tried, but no finite conclusion could be formed. Ultimately, it was believed there was no explanation for this seemingly inevitable loss of memory amongst the elderly. It was not until the beginning of the twentieth century that a breakthrough occurred. Alois Alzheimer examined the brain of a deceased individual who developed extreme memory loss during the later years of her life (Alzheimer's Disease, Marlene Targ Brill). Upon microscopic examination, it became clear that copious amounts of neurons had simply disappeared, leaving in their wake numerous protein aggregates that are known as senile plaques (Alzheimer's Disease, Marlene Targ Brill). Named for its discoverer, Alzheimer's Disease has continued to plague individuals for over one hundred years. These plaques, initially discovered in 1906, have become the hallmark characteristic of this neurodegenerative condition.

Still one of the most devastating diseases for the elderly population, Alzheimer's Disease affects approximately 30 million people worldwide, currently without a known cure (Heneka et al., 2007). As life expectancy constantly rises, the number of cases of Alzheimer's Disease will continuously increase, possibly tripling within fifty years (Heneka et al., 2007). Because of the extreme dementia associated with this disease, most of these patients necessitate some sort of care. Caregivers range from family members to

nurses specifically trained in working with the elderly. Once symptoms have become markably progressed, most patients eventually live in an assisted care facility or nursing home (Alzheimer's Facts & Figures). In America, costs for patients living in these facilities can range anywhere from $40,000 to $80,000 annually (Alzheimer's Facts & Figures). Due to the steep cost of assistance, the majority of individuals will spend their life savings over time, ultimately requiring financial aid through Medicaid services (Alzheimer's Facts & Figures). Affecting one in every six elderly individuals, it is estimated that 15 million Americans provide care to a relative or friend with this disease, totaling over $200 billion per year, creating an enormous, crippling strain on the national economy (Alzheimer's Facts & Figures).

Alzheimer's Disease is the sixth leading cause of death in the United States (Alzheimer's Disease Facts & Figures). In addition to national fiscal consequences, the task of caring for or providing care for a relative or friend with Alzheimer's Disease is usually placed on the family. Familial responsibility and care creates an entirely different set of problems. Roughly 15% of families live more than an hour away from their loved ones, resulting in a huge burden on the family, including higher costs for traveling to and from the patient (Alzheimer's Disease Facts & Figures). Not only are financial issues difficult for relatives to deal with, but the emotional toll on the family is taxing. Family members must witness their loved ones slowly lose their mental processes, causing emotions that make it hard to for the family to cope with their loved one's diagnosis. Today, one in three seniors dies of Alzheimer's Disease, illustrating just how prevalent this disease is in our society and how profound and far-reaching are its effects (Alzheimer's Disease Facts & Figures).

Typically characterized by gradual neuronal degradation within the hippocampus of the brain, Alzheimer's Disease has certain trademark characteristics (Sutcliffe et al., 2011). The loss of neurons in this region causes behavioral changes that can be observed in the patient. These transformations manifest themselves through memory deficiencies, ranging from mild, brief changes, to drastic long-term alterations. Cognitive deficits frequently begin with a loss of short-term memory; an inability to recall simple facts (Heneka et al., 2007). Events that occurred the day before become difficult to remember. This loss of episodic memory signals the initiation of Alzheimer's Disease symptoms. The gradual memory loss associated with Alzheimer's Disease slowly becomes more pronounced, progressing to the point where individuals become unaware of their own environment, believing that they, perhaps, are in a different time surrounded by different people. As if the mental changes are not enough, the individual's body begins to degrade as well, as the affected forfeits the ability to maintain control over their physical being (Heneka et al., 2007). Generally after the onset of these aggressive symptoms, the average lifespan is nine years (Heneka et al., 2007).

Much research has illustrated that these behavioral shortcomings are associated with significant changes in the adult Alzheimer's brain. These individuals display biological markers, and typically exhibit senile plaques characteristic of their condition (Jacobsen et al., 2006). These senile plaques consist of β-amyloid peptide aggregates, along with neurofibrillary tangles (Jacobsen et al., 2006). Neurofibrillary tangles generally consist of protein filaments in concert with hyperphosphorylated tau protein (Markesbery et al., 2010). Tau protein is important because it allows for stabilization of microtubules in the cell, facilitating cell to cell communication via molecular

transportation (Markesbery et al., 2010). Without proper regulation of tau protein, microtubules become insufficient and unable to correctly promote communication between cells. This destabilization ultimately prevents transport down a cell's axon, rendering neuronal communication inaccurate. Thus, a buildup of these neurofibrillary tangles with aggregates of amyloid-beta peptides causes a significant decrease in synaptic function and neuronal communication.

Due to its extreme importance in the progression of this dementia, amyloid-beta peptide has become the unique feature that differentiates Alzheimer's Disease from other neurodegenerative pathologies. Amyloid-beta peptide is synthesized through a complicated series of enzymatic reactions. The process of creating amyloid-beta is still somewhat poorly understood; however, the basic framework consists of multiple enzymatic cleavages. This chain of events ultimately results in the cleavage of amyloid precursor protein (APP) to the lethal amyloid beta protein associated with Alzheimer's Disease (Murphy et al., 2009). First, APP is cleaved by an enzyme known as β-secretase (Murphy et al., 2009). The resulting fragment is then quickly cleaved by gamma-secretase, creating different forms of β-amyloid peptide (Murphy et al., 2009). Along the way, various other activating proteins aid in the process, activating cleavage enzymes and permitting the creation of amyloid-beta peptide. The end of this pathway yields two differing forms of amyloid-beta peptide - Aβ-40 and Aβ-42. Although both are forms of amyloid-beta, they differ in the number of amino acids constituting the product. True, Aβ-40 is the more abundant species created; however, Aβ-42 tends to constitute the majority of the Aβ in the diseased brain, participating in and forming plaques in the brain that later lead to cognitive impairments (Murphy et al., 2009). The reason that Aβ-42 is

found in higher levels in the brain than its counterpart is that it has a much higher incidence of oligomerization (Mucke et al., 2007). Due to this trait, Aβ-42 has a higher probability of being misfolded and accumulating in the brain, resulting in large aggregations of Aβ-42 in the hippocampus.

Because it is one of many degenerative changes in the diseased brain, a lot of research has been done on the pathogenic effects of amyloid-beta peptide. In a review by Mucke et al. (2007), amyloid-beta is listed as one of the characteristic markers of Alzheimer's Disease, along with generalized atrophy and neurofibrillary tangles. An interesting fact noted in this review is the observation that although there are certain traits of the Alzheimer's brain, amyloid-beta peptide is consistently present, suggesting that there is an extremely high correlation, and possibly causation, between Alzheimer's Disease pathology and amyloid-beta peptide (Mucke et al., 2007). Further studies have indicated a potential mechanism for the deleterious results of excessive amyloid-beta accumulation. Long term potentiation (LTP) is the mechanism by which individuals form strong neuronal connections, creating memory. LTP endows individuals with the ability to learn tasks and commit them to memory. Studies have shown that amyloid-beta peptide accumulation has the ability to inhibit LTP (Mucke et al., 2007). When amyloid-beta antibodies were used against the peptide, LTP was not hindered, which would indicate that amyloid-beta has an effect on memory and cognition (Mucke et al., 2007). Because of these and other studies, amyloid-beta has been intimately linked with neuronal deficits seen in Alzheimer's Disease patients.

In addition to the buildup of neurofibrillary tangles and amyloid-beta plaques, microglial cells become overly activated, creating excessive inflammation (Heneka et al,

2007). Over activation of these protective immune cells results in the production of pro-inflammatory cytokines, ending in the initiation of an immune response. Unfortunately, current research suggests that the overactivity of microglial cells induces the production of more amyloid-beta peptide (Kahn et al., 2011). This, in turn, only furthers the immune response, causing more activation of microglial cells, and therefore more production of amyloid-beta peptide, suggesting a self-perpetuating causation of sporadic Alzheimer's Disease. Along with a general decrease in neuronal synapse formation, the extra production of amyloid-beta peptide creates pathologies that correlate with the clinical manifestations of Alzheimer's Disease.

Although amyloid-beta deposition is dangerous in all patients, there are certain individuals who carry more of a risk for development of Alzheimer's Disease. A large portion of those that become afflicted have certain pre-disposing genetic factors. These patients oftentimes develop early onset Alzheimer's Disease. In fact, most of the current research concerning amyloid beta production in the brain revolves around these patients. These traits manifest themselves in the form of mutations in one of three very specific genes, resulting in an overproduction of amyloid-beta peptides. One of the most dangerous mutations consists of a mutation in APP itself, creating a protein that has the potential to become hyperactive (Murphy et. al, 2009). In addition to this genetic malfunction, mutations in the enzymes cleaving APP can also prove harmful. Two proteins known as presenilin-1 and presenilin-2 are important because they are two subunits of gamma secretase, which is an essential enzyme in the production of amyloid-beta peptide. Mutations in the genes encoding presenilin-1 and presenilin-2 proteins have the potential to increase the production of amyloid-beta (Murphy et. al, 2009). Thus,

dysfunctions in either of these proteins can increase amyloid-beta production and deposition, resulting in familial early onset Alzheimer's Disease.

Along with genetic factors that influence the deposition of amyloid-beta peptides in the diseased brain, years of research have implied that the role of central inflammatory processes is just as important in the progression of Alzheimer's. Neuroinflammation in the central nervous system has been illustrated to correlate, if not initiate, the development of senile plaques (Ferretti et al., 2010). In fact, before the development of neurodegenerative plaques, pro-inflammatory cytokines are found in high concentrations in the brain (Ferretti et al., 2010). Along with these mediators, excessive amounts of amyloid-beta peptide are present within the brain, implying that there is a somewhat dependent relationship between the two events. Since high levels of these elements closely correlate with Alzheimer's Disease pathology, it has been hypothesized that preventing central inflammation could perhaps halt or delay Alzheimer's clinical manifestations. To this end, non-steroidal anti-inflammatory drugs were employed in patients with Alzheimer's Disease in the hopes that the disease would cease progressing or never present itself altogether (Ferretti et al., 2010). Although these studies were ineffective, it is possible that by the time the drug was given, the disease was too advanced, preventing any real benefit from the late usage of the drug.

Since many experiments have illustrated that amyloid-beta peptides are consistently found within the brain in Alzheimer's patients, various research groups have tried different ways to minimize its production. A study by He et al. (2010) was able to accomplish this feat, using a cancer drug known as Gleevec. Gleevec is used to treat stromal tumors and chronic myelogenous leukemia through inhibition of various tyrosine

kinases. One of its incidental, secondary effects is that inhibits cleavage and production of amyloid-beta peptide (He et al., 2010). In this study, He et al. showed how Gleevec can lower levels of amyloid-beta peptide. In the experiment, this group discovered a protein known as GSAP (gamma-secretase activating protein), which functions to activate gamma-secretase (He et al., 2010). Once gamma-secretase is active, it is able to cleave APP, initiating the pathway of production for amyloid-beta. Previous studies had explored the possibility of preventing cleavage of amyloid-beta peptide by attempting to inhibit amyloid-beta production at different steps in its creation. However, most of these studies were not successful, and those that were had deleterious secondary effects, rendering the therapies invalid (He et al., 2010). During this study, it was found that Gleevec was able to prevent GSAP activation of gamma-secretase, resulting in significantly lower levels of amyloid-beta in the brain, accompanied by the accumulation of less plaques in the hippocampus (He et al., 2010). One of the most important components associated with this study is the fact that deactivation of GSAP does not alter other important functions within the cell; rather, it only decreases the amount of amyloid-beta produced (He et al., 2010). Because Gleevec is unable to penetrate the blood brain barrier, He et al. theorized that a molecule that could cross the barrier and function to inhibit GSAP could be an effective therapeutic agent for Alzheimer's Disease.

Because of the important role of central inflammation in Alzheimer's Disease, scientists have begun to study the possible role of peripheral inflammation in the progression of the disease. Many of these initial studies have found that inflammation in the periphery may in fact exacerbate inflammation in the central nervous system. One such study reported that peripheral injections of a bacterial endotoxin, lipopolysaccharide

(LPS), resulted in increased levels of amyloid-beta peptides in the brains of mice (Lee et al., 2008). Although this study implied that peripheral inflammation does lead to higher levels of amyloid-beta in the mouse brain, unfortunately no behavioral analysis was conducted to explore the possible association of increased amyloid-beta peptide levels and cognitive deficits after prolonged exposure. To further explore this association, an even more recent study was done by our lab (Kahn et al., 2011). In this experiment, multiple peripheral injections of lipopolysaccharide were administered to mice over the course of one week. After seven days of peripheral injections, the amyloid-beta peptide levels in the mice brains were found to be significantly increased (Kahn et al., 2011). Along with this finding, the higher levels of amyloid-beta in the brain were correlated with an inability to learn hippocampus-dependent tasks (Kahn et al., 2010). Therefore, this study was able to conclude that peripheral inflammation created by LPS was able to induce elevated levels of amyloid-beta peptide in the hippocampus that corresponded with hippocampus-dependent behavioral shortcomings. Because of the correlation between these behavioral deficits and elevated levels of amyloid-beta peptide in the brain, it was hypothesized that amyloid-beta might be responsible for the hippocampal learning deficiencies viewed in the LPS-injected mice.

The increased interest in peripheral inflammation in the role of Alzheimer's Disease spurred many more important studies, including one by Sutcliffe et al. (2011). In this novel study, it was illustrated that the amyloid-beta peptides constituting the plaques in the brain most likely originate from the periphery, specifically the liver. From the study done by He et al. (2010), the researchers knew that Gleevec was able to inhibit production of amyloid-beta peptide via interactions with GSAP. Additionally it was

known that Gleevec itself does not have the ability to penetrate the blood brain barrier. Therefore, if Gleevec was given peripherally to wild-type mice and levels of amyloid-beta peptides in the brain significantly decreased, the most likely origin of the peptide would be the periphery. In the experiment, wild-type mice were given peripheral injections of Gleevec twice daily for two weeks (Sutcliffe et al., 2011). After injections were completed, samples of plasma and brain tissue were collected and tested for amyloid-beta peptide. The results indicated that there were significantly low levels of amyloid-beta peptide not only in the periphery, but in the brain as well (Sutcliffe et al., 2011). Because Gleevec does not possess the ability to cross the blood brain barrier, this study suggested that the majority of amyloid-beta peptides in the brain are of peripheral origin, opening up new possibilities for treatment.

The present study investigates the idea that amyloid-beta is the peptide responsible for the previously noted hippocampus-dependent deficiencies. Using LPS injected in the periphery of mice, we were able to induce the creation and deposition of amyloid-beta peptide in the hippocampus. Combining LPS and Gleevec injections in a different group of mice allowed us to visualize that amyloid-beta levels in the hippocampus were significantly lower than in the mice that did not receive Gleevec treatment. After two weeks of injections, animals were then subjected to Contextual Fear Conditioning (CFC). CFC associates a context (or contexts) with an electric shock, with the goal that animals who have normal hippocampus function will be able to remember the shock when they see or smell the context in which the shock was administered. Mice that remember this association will freeze, and refrain from making a lot of movements. After two days of CFC, the amount of time animals spent freezing was analyzed, and

mice brains were examined for levels of amyloid-beta peptide. The results of this experiment revealed that animals who received Gleevec had significantly lower levels of amyloid-beta peptides in the hippocampus. Additionally, these animals were able to correctly learn hippocampus-dependent tasks as assessed in CFC. These results suggest that amyloid-beta is most likely responsible for the hippocampus-dependent deficits observed in our mice, suggesting that blocking amyloid-beta production in the periphery would be sufficient for a possible therapeutic treatment for Alzheimer's Disease.

MATERIALS AND METHODS

Subjects and Housing

Ranging from 4-6 months old, male C57BL/6J mice were used in this study. These mice were bred in the TCU vivarium, originating from a breeding colony located in Jackson Laboratory in Bar Harbor, Maine. These animals were cared for according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010), as well as by procedures outlined by the Institutional Animal Care and Use Committee (IACUC) of Texas Christian University. Animals were housed in cages measuring 12.5 cm x 15 cm x 25 cm. Each cage held a group of three to four animals. Cages remained in the same area throughout the experiment. Daily rhythms were kept, and lights were on at 700 AM and turned off at 700 PM. Control and experimental groups were subject to the same living conditions.

Biological Assays

Treatments

Intraperitoneal injections (i.p.) were administered to subjects. Injections consisted of 20mg/kg Gleevec (IM) or volume-equivalent saline. Saline and Gleevec injections

were given twice daily for a total of fourteen days (days 1-14). Beginning the second week, a single injection of 250μg/kg LPS (*Escherichia coli* serotype: 055:B5; Sigma-Aldrich, St. Louis, Missouri) or saline was additionally given. These treatments created four groups: Gleevec-saline, Gleevec-LPS, saline-saline, and saline-LPS (see Figure One).

Cytometric Bead Array

At the start of the second week of injections, animals received a single LPS injection. Four hours after receiving this single injection, blood was obtained from the tail veins of the subjects. Peripheral pro-inflammatory cytokines and chemokines levels were measured from the serum. Pro-inflammatory cytokines studied included IL-6 and TNF-α, while chemokines consisted of MCP-1 and MIP-1 α. Chemokines and cytokines were measured via cytometric bead array (CBA; BD Biosciences, San Jose, California), following kit instructions and procedures. Data was then collected on a FACSCalibur flow cytometer (BD Biosciences, San Jose, California) using CellQuest Pro software (BD Biosciences). Data was next analyzed utilizing FCAP Array software (Soft Flow, Inc., New Brighton, Minnesota).

Tissue Preparation

After two weeks (14 days) and completion of injections and CFC, mice were euthanized via CO_2 inhalation, in accordance with IACUC. Hippocampal tissue was then instantly removed and prepared for analysis. The analysis began with tissue preparation for a protein assay and amyloid-beta ELISA. The ELISA procedure necessitated homogenization of brain tissue with protein extraction solution (PRO-PREP, Boca Scientific, Boca Raton, Florida). Protein extraction solution contained protease inhibitors

which interacted with the hippocampal tissue. Samples were placed on ice for 30 minutes, then stored overnight at -80°C. After storage, these mixtures were centrifuged at 16,000 x g for 30 minutes. The purified samples were then ready for *DC* Protein Assay (Bio-Rad Laboratories, Hercules, California), followed by $A\beta_{X-42}$ ELISA (Covance Research Products, Dedham, Massachusetts).

Amyloid-Beta Procedure

BetaMark $A\beta_{X-42}$ ELISA (Covance Research Products, Dedham, Massachusetts) was then carried out to measure levels of amyloid-beta peptide in the hippocampus. Our ELISA was conducted following instructions from the kit itself. Essentially, we made an incubation buffer as well as standard intermediates. Our unknown samples were then diluted with buffer. On each well of the ELISA plate was an antibody. Once our samples were prepared, they were added to the wells of the ELISA plate in addition to the standards. The standards we created were then used to create a standard curve, with which we compared the values of our unknowns. All results were read on a multi-well spectrophotometer at 620nm (BMG LabTech FLUOstar Omega, Cary, North Carolina).

Behavioral Paradigms

Contextual Fear Conditioning

Contextual Fear Conditioning (CFC) used automatic fear conditioning chambers (Coulbourn Instruments, Whitehall, Pennsylvania, 7Wx7Dx12H) and FreezeFrame™ software (ActiMetrics Software, Wilmette, IL). The chamber and software together allowed for the measurement of freezing behavior. Each chamber contained a grid floor, providing a means for the electrical, adverse stimulus to be administered. At the top of the chamber was a camera that monitored all the movements of the animals, allowing

measurement of freezing behavior. Along with a shock, dotted walls and an olfactory cue were utilized as the contexts. CFC training began 24 hours after the final injection of either LPS or saline, followed by testing an additional 24 hours after training. A 120 second acclimation period began the training session. After this was complete, a 2 second 0.7mA shock was administered. Once the stimulus was delivered, animals lingered in the boxes for another 60 seconds. During testing day, there were no shocks. Animals were placed in the boxes and animal movements and freezing behavior were monitored via the computer system for 90 seconds. The amount of time in seconds animals spent freezing was then collected and analyzed.

Statistics

We used variance analysis (ANOVA). This program allows determination of significant difference between the groups. Once significant difference is found, Fisher's PLSD post-hoc tests are utilized to determine the experimentally different groups.

RESULTS

Gleevec does not prevent LPS-induced inflammation

To prevent confounding of results, it was essential to show that Gleevec itself does not prevent LPS-induced inflammation from occurring. To this end, pro-inflammatory cytokines and chemokines were measured four hours after a single LPS injection. Animals who received Gleevec injections or saline injections for seven consecutive days received one intraperitoneal LPS injection. We measured cytokines IL-6 and TNF-α and chemokines MCP-1 and Mip-1α. Our results show that levels of pro-inflammatory cytokines and chemokines were significantly elevated in both treatment groups. This data demonstrated that inflammation was present in both groups.

Additionally, levels of pro-inflammatory cytokines and chemokines were significantly increased in animals who received a single LPS injection after saline for seven days, as well as for animals who received the same single LPS injection after administration of Gleevec for seven days (for IL-6 ($F_{(1,28)}$= 35.984, $p<0.0001$), TNF-α ($F_{(1,28)}$= 16.304, $p<0.01$), MCP-1 ($F_{(1,28)}$= 51.152, $p<0.0001$), and Mip-1α ($F_{(1,28)}$= 6.524, $p<0.05$). Further, using Fisher's PLSD post-hoc analysis, we saw that there was no significant difference in pro-inflammatory cytokines and chemokines between subjects receiving saline or Gleevec ($p>0.05$), suggesting that Gleevec does not significantly increase the inflammatory response. These findings are imperative because they prove that Gleevec alone does not interfere or inhibit the production of pro-inflammatory cytokines or chemokines. Therefore, other explanations for a reduction in amyloid-beta peptide in the brain, such as an inhibition of the inflammatory response by Gleevec, are invalid.

Gleevec reduces LPS-induced hippocampus amyloid-beta concentration

To illustrate that peripheral injections of Gleevec cause a reduction in LPS-induced amyloid-beta peptide peptides in the hippocampus, it was necessary to measure amyloid-beta levels. We used an $A\beta_{x-42}$ ELISA to measure these levels. The ELISA results demonstrated that Gleevec did in fact reduce amyloid-beta production as measured by the amount of amyloid-beta peptides found in the hippocampus. Our data illustrates that animals receiving LPS or saline showed significant differences in amyloid-beta hippocampal levels ($F_{(1,36)}$= 15.143, $p<0.001$) when compared to the animals receiving Gleevec or saline ($F_{(1,36)}$=5.435, $p<0.05$). The final results indicated that the interaction between the two components were influential in amyloid-beta production ($F_{(1,36)}$= 6.577, $p<0.05$). When animals were injected with saline-saline and Gleevec-

saline, as expected there was no increase in amyloid-beta in the hippocampus. However, when animals were injected with LPS-saline, there was a significant increase in hippocampal amyloid-beta discovered in the mouse brain ($p < 0.05$) when compared to those mice that received Gleevec-LPS. Using Fisher's PLSD post-hoc analysis, we were able to see these results. The above data indicate that Gleevec is indeed effective in reducing amyloid-beta compilation in the hippocampus, probably through peripheral interactions.

Gleevec restores cognition

In addition to discerning that Gleevec inhibits buildup of amyloid-beta peptide in the hippocampus, we were able to prove that Gleevec given in concert with LPS allowed restoration of hippocampus dependent behavioral deficiencies. By measuring freezing behavior in CFC on training and testing day, we were able to ascertain that there was no difference in condition in LPS or saline ($F(1,36)=0.134$ *NS*), treatment with Gleevec or saline ($F(1,36)=3.314$ *NS)*. Also, there were not any interactions between condition and treatment ($F(1,36)=0.839$, *NS*). Since there were no statistically significant differences on training day, we knew that between our four groups, there were no significant behavioral anomalies. However, after measuring time spent freezing on testing day, there were significant differences when compared to training day. These were observed through treatment with either LPS or saline ($F(1,36)=9.173$, $p < 0.01$) or condition of Gleevec and saline ($F(1,36)=5.399$, $p < 0.05$). Together, the interaction between treatment and condition revealed significant behavioral differences ($F(1,36)=4.251$, $p < 0.05$). Previous analyses as stated above show that the saline-LPS animals demonstrated a significant cognitive decline as opposed to the other three groups of animals ($p < 0.05$). However,

animals given Gleevec-LPS showed a restoration of cognitive function, revealing that there was no significant difference in cognitive function between this group of animals and those given saline-saline or Gleevec-saline. These results could signify that LPS-induced hippocampus deficits may be secondary to extreme buildup of amyloid-beta in the hippocampus.

DISCUSSION

In the present study, we sought to determine whether or not previously observed hippocampal dependent learning deficits were due to the accumulation of amyloid-beta peptide in the brain. Our lab has previously shown that peripheral injections of LPS result in elevated levels of amyloid-beta in the hippocampus, as well as visible cognitive deficits, observable through CFC testing (Kahn et al., 2011). From a prior study conducted by He et al. (2010), we know that Gleevec is able to limit production of amyloid-beta peptides via inhibition of GSAP, an activating molecule essential in the production of amyloid-beta. Through multiple studies reviewed by Selkoe et al., (2007), amyloid-beta has been illustrated to have extremely deleterious effects on cognition, especially longterm potentiation. Interfering with LTP provides a mechanism by which amyloid-beta peptide could be related to observed Alzheimer's Disease behavior. In another study by Sutcliffe et al. (2010), it was shown that the most likely origin of amyloid-beta in the hippocampus is the periphery, more specifically the liver. This would suggest that the majority of amyloid-beta in the hippocampus is transported into the brain from the periphery. Therefore, we proposed that utilizing a known amyloid-beta inhibitor in concert with peripheral LPS injections would allow us to see if the observable

cognitive deficits viewed in CFC testing were attributable to the amyloid-beta buildup in the hippocampus.

To test our hypothesis, we used four experimental groups of mice that received injections twice daily (saline-saline, Gleevec-saline, saline-LPS, Gleevec-LPS). Our prediction was that the mice who received Gleevec along with LPS would show significantly lower levels of amyloid-beta peptide in the hippocampus when compared to animals who received solely LPS. Because our lab has illustrated that elevated amyloid-beta peptide levels are correlated with hippocampus dependent behavioral deficits, we reasoned that mice with lower hippocampal levels of amyloid-beta in the brain would exhibit essentially no cognitive deficits. If cognitive restoration is observed, then we could reasonably conclude that the learning deficits are attributable to amyloid-beta buildup in the hippocampus.

To prevent confounding of results, it was essential to show that Gleevec itself does not prevent inflammation from occurring. We gave injections of Gleevec to test subjects for fourteen days. If restoration of cognitive function was observed in mice receiving Gleevec in concert with LPS, a possible explanation could be that Gleevec did not restore cognition; but rather, it prevented any inflammation from happening. To counteract this alternative conclusion, blood was taken from mice four hours after receiving their first LPS injection to test for pro-inflammatory cytokines and chemokines. These molecules served as an indicator, enabling us to see if inflammation was in fact present in the Gleevec treated mice. As expected, levels of IL-6, TNF-alpha, Mip-1α and MCP-1 were elevated in the groups of mice who had received saline-LPS for seven days, as well as in those who had received Gleevec-LPS for seven days. Another caveat to this

experiment would be the possibility that Gleevec in fact promoted an increased inflammatory response. In other words, if this was true, we could expect to see significantly higher levels of pro-inflammatory mediators in animals injected with Gleevec-LPS when compared to animals injected with saline-LPS. However, we observed that there was no significant difference in levels of pro-inflammatory cytokines and chemokines, making it highly unlikely that Gleevec exacerbated an inflammatory response. These results showed that an inflammatory response had indeed occurred in all animals. This would suggest that our resulting data would not be due to Gleevec simply inhibiting the inflammatory response.

After completion of injections, it was necessary to assess behavior. Utilizing CFC training and testing, our lab was able to measure freezing behavior. In order for our behavioral results to be accurate, there needed to be no significant difference between animal behavior in any group on training day. Our results indicated that there was in fact no significant difference between animal freezing behavior during training, meaning that any differences we saw on testing day would not be attributable to pre-existing cognitive issues or sickness behavior, both of which could confound learning. After CFC was complete, our results indicated that, as we hypothesized, animals who received Gleevec-LPS demonstrated a restoration of cognitive function. To correlate our behavioral findings with levels of amyloid-beta in the hippocampus, we used an ELISA to measure levels of amyloid-beta peptide. As expected, we discovered that levels of amyloid-beta in the hippocampus of mice receiving Gleevec-LPS were significantly lower than those animals who received saline-LPS.

Using Gleevec to block amyloid-beta accumulation in the hippocampus, we were able to determine whether or not the hippocampus deficits previously noted were indeed due to amyloid-beta accumulation. Our results indicate that mice receiving Gleevec-LPS did actually have lower levels of amyloid-beta peptides in the hippocampus. To accompany this, the mice receiving the Gleevec-LPS treatment showed a restoration of cognition, meaning that these mice were able to learn. These results would suggest that amyloid-beta is the peptide responsible for the observed hippocampus dependent behavioral shortcomings. Another important facet is that Gleevec has been shown to poorly penetrate the blood brain barrier. Although inflammation could possibly alter the blood brain barrier such that Gleevec is able to enter the brain, our results illustrate that Gleevec would indeed still be effective in preventing amyloid-beta accumulation in the hippocampus. However, since we believe that Gleevec mainly functions in the periphery, based on our results it is reasonable to conclude that at least some of the amyloid-beta buildup in the hippocampus originates in the periphery. True, no study was directly conducted to determine that the amyloid-beta peptides found in the brain definitively come from the periphery; however, our results indicate that this could be a possibility, and should be an area for further exploration. Our findings suggest that peripherally given LPS leads to higher levels of amyloid-beta in the hippocampus, resulting in an inability to learn hippocampus dependent tasks. These results imply that amyloid-beta peptide could be a possible target for Alzheimer's Disease therapy.
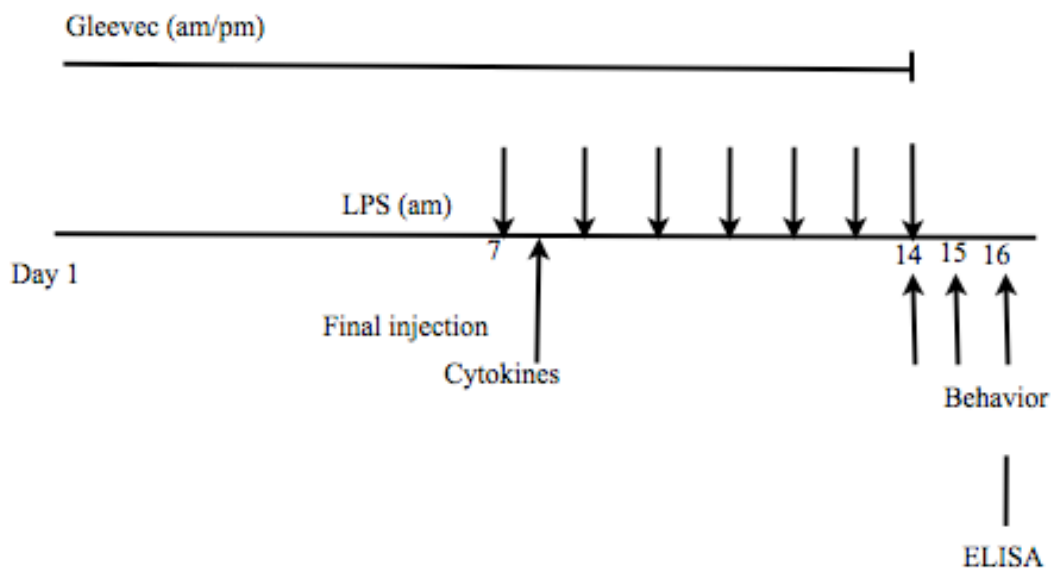
Figure One

Figure 1. Experimental Procedure. Starting Day 1, animals were given either saline or Gleevec twice daily for one week. Beginning the second week, a single LPS injection was given. Four hours after this injection, pro-inflammatory cytokines and chemokines were measured. On Day 14, animals received their last injection. Day 15 began CFC testing, which was concluded by Day 16. At this time, animals were euthanized and an amyloid-beta peptide ELISA was conducted to measure peptide levels.
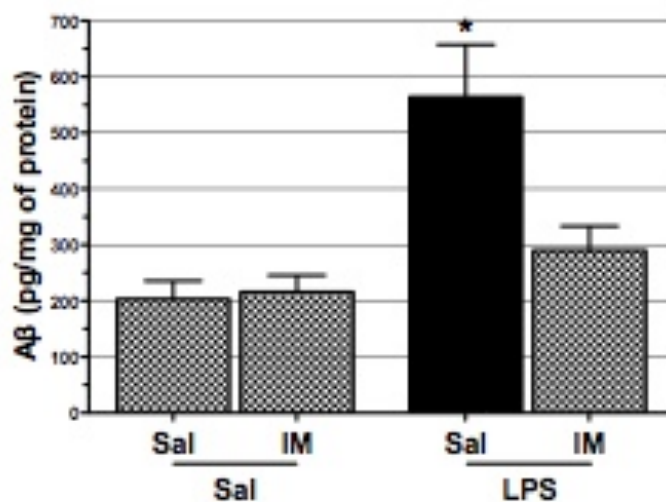
Figure Two

Figure Two. Amyloid-beta levels following co-administration of Gleevec-LPS. LPS-induced amyloid-beta production is prevented by Gleevec (IM) co-administration. After seven consecutive days of LPS treatment, amyloid-beta is significantly elevated in the mouse hippocampus ($p<0.05$). Administration of LPS and Gleevec prohibits the elevation of amyloid-beat in the mouse hippocampus.
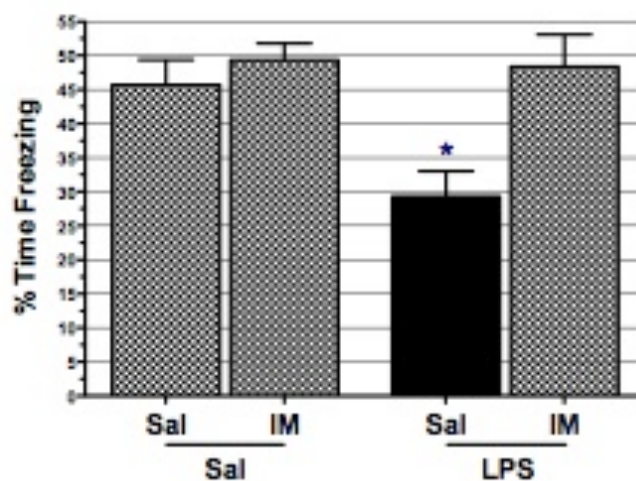
Figure Three

Figure 3. Cognitive deficits as observed in CFC. LPS-induced deficits in cognition are restored following Gleevec administration. After one week of LPS treatment, mice behavior in contextual fear conditioning is significantly impaired (p<0.05). Co-administration of Gleevec and LPS restores and rescues cognition. Bars represent +/- SEM.
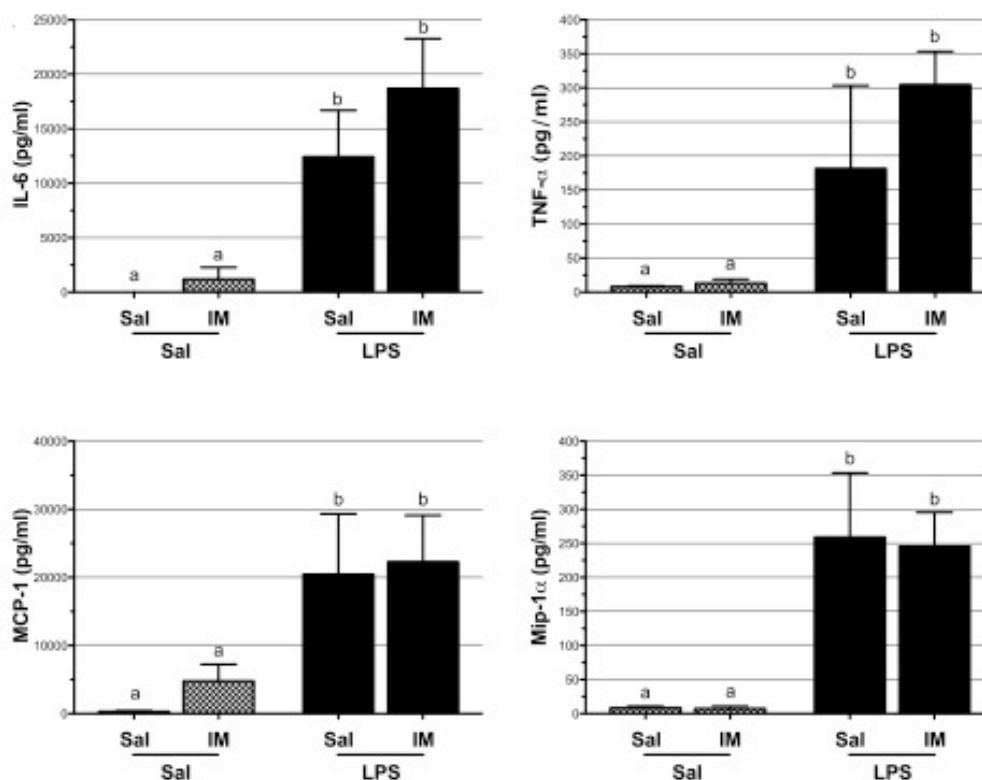
Figure Four

Figure Four. Gleevec does not inhibit inflammatory response. Gleevec (IM) does not prevent LPS-induced inflammation. One of the important facets of this experiment was illustrating that Gleevec itself does not hinder inflammation from occurring. Animals were given saline or Gleevec for seven days, followed by a single LPS injection. In order to show that the week of Gleevec injections did not hiner inflammatory processes, we measured pro-inflammatory cytokines (IL-6 and TNf- α) and chemokines (MCP-1 and MIP-1α) four hours after initial LPS injection. "a" and "b" groups denote significant differences ($p<0.05$) in levels of pro-inflammatory cytokines and chemokines. Bars represent +/- SEM.

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease characterized by atrophy of the adult brain and increased presence of extracellular amyloid-beta (Aβ) plaques and intracellular tau tangles. Presence of these truncated or misfolded proteins results in altered cellular signaling, oxidative stress, and ultimately to cell death. Chronic inflammation has been implicated in the onset and progression of these AD pathologies. Previous studies in our lab have shown that peripheral inflammation can lead to increased central Aβ and deficits in learning and memory. In order to determine if the Aβ accumulation in the brain is responsible for the learning deficits, we attempted to decrease peripheral production of Aβ in order to reduce central Aβ accumulation. It has previously been shown that Aβ is produced in large quantities in the liver, and is transferred across the blood brain barrier. Recent research has shown that peripheral treatment with imatinib methanesulfonate salt (IM), a form of the anti-cancer drug Gleevec™ and known to interfere with the interaction between gamma-secretase and the gamma-secretase activating protein (GSAP), decreases the cleavage of peripheral amyloid precursor protein into Aβ. Because IM poorly penetrates the blood brain barrier, we hypothesized that co-administration with LPS would decrease peripheral production of Aβ leading to a decrease in Aβ accumulation in the hippocampus. We show here that peripheral IM treatment eliminates the elevation in hippocampal Aβ following LPS-induced peripheral inflammation. More importantly, IM eliminates the cognitive impairment seen following 7 consecutive days of LPS administration, implicating Aβ peptides as the cause of these performance deficits.