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Rylan M. McQuade
Southeastern University - Lakeland

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The Therapeutic Role of Turmeric in Treatment and Prevention of Alzheimer’s Disease

Rylan M. McQuade

Department of Natural Sciences. Southeastern University, Lakeland, Florida, USA
Abstract: As a devastating neurological condition that expends millions of lives each year, Alzheimer’s disease (AD) is a subject of intense investigation. Although AD has been known for over a century, the precise mechanisms that underlie AD pathogenesis and development are still poorly understood. The Alzheimer phenotype is typified by extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs), causing researchers to notice several key enzymes implicated in this process. Most notable are β and γ secretases (which drive Aβ plaque production) and phospholipase A₂ (which stimulates major cascade activation through the specific cleavage of fatty acyl esters). Both acidic and neutral sphingomyelinases of the ceramide pathway are also strongly linked to AD development and progression. As a specific type of esterase, PLA₂ specializes in phospholipid catabolism by specifically cleaving fatty acyl bonds at the sn-2 position, producing a free fatty acid and lysophospholipids as products. This stimulates the arachidonic acid (AA) cascade, while lipid cleavage by sphingomyelinase upregulates the ceramide pathway. Cyclooxygenase (COX) and lipooxygenase (LOX) are suspected to be key players in oxidative and neurological damage and are involved in both these metabolic pathways. A further investigation of these molecular messengers and their cellular environments is absolutely essential for therapeutic intervention and effective medical treatments. Recently, the role of herbal medicines has been investigated to produce possible leads for Alzheimer’s treatment. Turmeric contains over 20 medicinally useful compounds, of which curcumin is a key component. In this review, we hypothesize that turmeric will prove especially useful in preventing amyloid-β plaque deposition in early stages of AD and improvement of learning and coordination in middle stages of the disease. Further evidence suggests curcumin’s efficacy in resisting ROS, preventing tau aggregation, and limiting the spread of pro-inflammatory molecules throughout the body. Because of its highly beneficial effects as an Alzheimer therapeutic, we commend that turmeric be studied in greater detail and incorporated into Western medical treatments. Additionally, a prospective study will be presented to determine the efficacy of turmeric therapies, both in improving physical activities and overall neurological health. Although turmeric’s precise mechanisms are not yet understood, multiple studies have confirmed its beneficial effects in preventing amyloidogenesis by modulating esterase and sphingomyelinase activities and also resisting misdirected
cleavage of APP. Turmeric has also been found to downregulate abnormal GSK-3β function and prevent formation of SPs and NFTs. Interestingly, murine models subjected to a turmeric regimen not only displayed a reversal of AD symptoms, but also demonstrated a lower susceptibility to neurodegenerative disease. Collectively, these findings strongly support turmeric’s therapeutic use in Western medicine.

Chapter 1

Introduction

As a widespread ailment that affects millions worldwide, AD wrecks a deadly impact on the elderly population. Recently, the Western world has experienced a dramatic peak in Alzheimer’s incidence. In this year alone (2015), over five million Americans are currently living with AD. Certainly, Alzheimer’s is nothing new. Cases of pre-senile dementia have been reported from early Mesopotamia and ancient Egypt when external manifestations of the disease were noticed without much (if any) understanding of its inner workings. Recently, however, AD has struck with a dogged intensity to become one of the leading causes of death among Western cultures. What are the reasons for AD’s sudden appearance? How does this disease begin and what are its hallmark symptoms? Genomic comparisons and intensive genetic research reveal that humans have always possessed the potential for AD development. Indeed, AD pathology seems to be permanently etched on the Homo sapiens genome, consistently conserved from one generation to the next. Because of the body’s intrinsic ability to produce β and γ secretases, anyone can develop insoluble plaque deposition when secretases are overstimulated. Similarly, every individual has the ability to develop NFTs from excessive tau protein phosphorylation. With improved living conditions and medical treatment, the geriatric population has increased substantially in recent years and correlates closely with increasing AD incidence. AD largely affects the elderly, usually 65 years or older.

Alzheimer’s disease progression is split into three overlapping categories: First, the subject finds it difficult to remember recent events. This period usually lasts around two to
four years and is typified by mild bouts of confusion concerning daily routine and activities. Next, the patient enters a stage of persistent confusion, often forgetting the identities of close friends and family members. This part of disease progression is especially devastating since it often robs the patient of his or her self-identity as neuronal necrosis occurs more rapidly. Finally, the individual enters a sudden onset of physical and cognitive decline, ultimately leading to death. Disease progression is a prolonged and bitter defeat, stripping away memories and leaving feelings of social isolation and a lost personal identity in their place. Countless therapies have been developed for AD, ranging from COX-2 inhibitors to more progressive drugs like memantine and NSAIDs. Despite displaying some promise, these drugs are largely inefficient in addressing the root cause of AD pathology. The failure of modern AD therapies has driven researchers to delve further, searching for the most effective treatment. Recent studies suggest that turmeric may possess the clinical and pharmacological effects that reverse AD symptoms while also preventing disease pathology. This chapter will review AD’s enormous impact on the Western world, the rising cost of research, and the lipase enzymes involved in driving amyloidogenesis. It will be shown precisely how enzymes like secretases and PLA2 directly impact AD development by producing Aβ peptides and pro-inflammatory molecules respectively.

**Alzheimer’s and the Western World**

Due to the rising cost of neuropathological research and increasing incidence of neurodegenerative diseases, much of current research is turning to homeopathic remedies, largely derived from plant-based materials. The use of turmeric corresponds with many aspects of Adurvedic (Indian) medicine and is frequently used in tandem with additional Adurvedic compounds, such as *Convolvulus pluricaulis* (CP), liquiritin (obtained from liquorice), and shankhpushpi (gathered from the alowe weed). Despite its frequent medicinal use in much of Eastern medicine and its promising neurological benefits from multiple studies, turmeric (to this date) is still not used therapeutically in Western medicine. As researchers are desperately screening novel compounds and developing
molecular analogues for the treatment of AD, turmeric (specifically curcumin) must be examined more thoroughly as a potential candidate for AD medicines. Before turmeric can be investigated in further detail, however, the basic mechanisms underlying AD must be presented and understood.

As a fatal neurological disease that expends millions of lives each year, AD is a major health concern among developed countries. It is especially prevalent across North America and Europe, causing significant health concerns worldwide. In Western cultures, individuals of 65 years exhibit an AD occurrence of 8%; this figure escalates dramatically with age, affecting over 45% of 85-year-olds. With increasing medical costs and rising expenditure of drug screening, many researchers are searching for simpler, more efficient alternatives. Rather than halting disease conditions, current treatments are merely palliative, hoping to ease symptoms in spite of internal tissue necrosis. In this regard, even the most developed therapeutics are incompetent to prevent AD pathogenesis and development. Researchers are desperately searching for a cure but so far have been left without any conclusive results. It seems that the search for designing an effective AD drug regimen has all but exhausted its efforts and research is turning to components of Eastern medicine that exhibit curative potential. As a key medicinal compound and major dietary component in Eastern cultures, turmeric may be useful in preventing the pathogenesis of AD, as well as easing symptoms. After presenting relevant studies and outlining current understandings of the disease, the efficacy of turmeric (specifically curcumin) will be discussed and evaluated. First, a comprehensive overview of AD—its hallmark symptoms, its molecular and genetic origins within the body, and molecular inhibitors to stop AD progression—will all be presented and discussed in greater detail. Finally, turmeric’s novel properties and its exceptional use as a dose-dependent therapeutic will be presented and examined.

**Basics of AD**

A devastating and dehabilitating illness, AD is the subject of intense investigation. This disease is becoming increasingly common with millions of cases each
year and exorbitant costs expended in treating the disease. A chronically progressive category of dementia, AD drastically impacts memory and spatial learning capabilities. Patients diagnosed with AD typically survive only three to nine years following AD onset, manifesting both physical and social deterioration. Although the subject of exhaustive research, the exact cause of AD still remains uncertain. Approximately 5% of cases are suspected to be genomically linked and are subject to the influence of many polygenomic sequences. Other cases are presumed to be environmental, either linked to diet or lifestyle choices that are detrimental to overall homeostasis. Understandably, head trauma can lead to neural disruption, but more obscure factors (such as high blood pressure) can easily form a complex web of AD’s causative factors. Alzheimer’s complex ontology coupled with its multiple phenotypic effects and interlinked signaling cascades provides a difficult scenario for researchers. Each new finding underscores the conviction that AD is a multifactorial illness, stimulated by a host of complex molecular interactions. Discovered over a century ago by Alois Alzheimer—a Bavarian psychiatrist and neuropathologist—the mysteries behind AD remain enigmatic. Originally categorizing the disease as “pre-senile dementia,” it was later termed “Alzheimer’s disease” by Dr. Emil Kraepelin. AD typically afflicts elderly patients (65 years or older), although pre-senile dementia in younger patients is also a well-documented phenomenon. As mentioned earlier these pre-senile cases are often linked to genetic abnormalities or to head trauma early in life. Alzheimer’s disease progression is split into seven distinct stages, ranging from pre-onset of disease symptoms to severe cognitive, social, and cellular dysfunction.

As the most common form of dementia, AD patients typically display signs of forgetfulness and short-term memory (STM) impairment. Over the years, symptoms progressively worsen until the patient displays dramatic behavioral changes, motor/kinetic imbalance, and gradual loss of coordinated movement. Left untreated, the patient deteriorates and death inevitably follows within three to nine years. In nearly all cases, AD onset is associated with internal neurofibrillary tangles (NFTs) and external senile plaques (SPs). NFTs are mainly composed of excessively phosphorylated tau protein, which is overexpressed and has become insoluble in the intracellular

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environment. This is most likely due to erroneous expression of the tau gene, causing protein overexpression and misfolding. Alteration of protein configuration mainly occurs when defects occurring in the protein’s 3-dimensional structure are not corrected by molecular chaperones. Because of this buildup of misfolded proteins, synapse degradation is a common phenomenon of most tauopathies. As neural junctions become clogged and the axon and cell body become crowded with proteinaceous debris, neural tissues deteriorate and the overall health of the patient decreases. Under normal conditions, tau proteins help support microtubules and facilitate molecular transport across the axon. In AD pathology, tau aggregates to form large secondary structures called paired helical filaments that become tangled and interwoven, causing impairment of neuronal functions.

Contrastingly, SPs are mainly present extracellularly and consist almost entirely of amyloid-β (Aβ) peptide. Ultimately, Aβ peptides originate from the APP gene, which encodes the amyloid protein precursor (APP). Although APP is the substrate in a myriad of enzymatic reactions, it is largely cleaved through the actions of three main secretase enzymes—α, β, and γ. In multiple studies, α-secretase has been noted for its beneficial effects involving neural growth and protection. A disintegrin and metalloprotease domain protein (ADAM), α-secretase is normally found near the cell glycocalyx or imbedded as a transmembrane protein within the phospholipid bilayer. α-secretase cuts APP (using ADAM10 and ADAM17) which creates the C-terminus (C83) portion and the secretion of larger α APP, which collectively regulate brain function. The C83 part is retained within the lipid bilayer where it is enzymatically cleaved by γ-secretase, producing a P3 peptide. Although found mainly in the plasma membrane, ADAM10 is also found in significant amounts in the Golgi apparatus. When a transmembrane protein is cleaved so that its extracellular domain is freed, this is called “ectodomain shedding.” In contrast to α-secretase, β- and γ-secretases are known to stimulate the production of Aβ_{1-40} and Aβ_{1-42} oligopeptides, which are equally implicated in AD development. In AD pathogenesis, β-secretase (BASE1) acts upon APP first, cleaving APP at its N-terminus by a catalytic aspartate residue in its active site. This produces sAPPβ and a C-terminal fragment (CTFβ) called C99 is produced. sAPPβ is
then cleaved by \( \gamma \)-secretase at the C-terminus to make multiple A\( \beta \) oligopeptides.\textsuperscript{25} Studies overwhelmingly demonstrate that A\( \beta \)\textsubscript{1-40} and A\( \beta \)\textsubscript{1-42} are most detrimental to neural health and are key players in AD pathology. Although originally soluble, A\( \beta \) plaques eventually aggregate to assume their insoluble form.\textsuperscript{26} This is due to steric considerations involving surface area, as well as intramolecular interactions that make the aggregates insoluble. Although soluble polymers have been the main subject of concern, recent research suggests that soluble plaques may be equally (if not more) deadly than the insoluble form.\textsuperscript{27} Although the precise details behind their neurotoxicity still remain a mystery, researchers have found that soluble (s) A\( \beta \) peptides exert a lethal impact on miRNA, exerting their efforts through N-methyl-D-aspartate (NMDA) receptors and through reactive oxygen species (ROS).\textsuperscript{28} This finding highlights the need for total prevention of A\( \beta \) peptide production, both soluble and insoluble forms.

A portion of \( \gamma \)-secretase (presenilin-1) is a central component for APP’s activity. Researchers have found that even slight structural changes in presenilin-1’s structure are linked with familial AD (which appears early in life).\textsuperscript{29} Since A\( \beta \) plaque detection is often obscured by confounding factors, AD diagnosis is often difficult and SPs are usually observed in deceased specimens. Several methods, however, exist for AD detection \textit{in vivo}. Positron emission tomography (PET) allows recording of abnormalities in glucose metabolism, while MRIs can relay shrinkage of neural tissue that is noticeably smaller than healthy brain tissue.\textsuperscript{30} Behavioral studies reveal that AD primarily affects hippocampal functions involving memory storage and the entorhinal cortex (a processing portion of the temporal lobe). Further investigations reveal that TNF-\( \alpha \) may indirectly encourage AD development.\textsuperscript{31} TNF-\( \alpha \) converting enzyme (TACE) is a type of ADAM whose activity liberates TNF-\( \alpha \) into the extracellular environment. These proteins usually contain large amounts of cysteine residues, which are implicated in joining of cells and protein aggregation caused by disulfide bonding. In recent years, tumor necrosis factor (TNF) \( \alpha \) is a subject of interest for researchers. As a master-regulator of the immune system, it is involved in critical reactions involving bodily defense systems, carcinogenesis, inflammation, and regulated cell death.\textsuperscript{32} It is mainly secreted by macrophages that have been alerted to exogenous threats to immunity. The immune
system is well known for its rapid response to exogenous threats and external insults to the body. An invading bacterium, a virus, or even an epidermal abrasion are common stimulatory factors of the immune response.\textsuperscript{33} The immune system’s control of endogenous systems, however, is only beginning to be understood. Indeed, science has only touched the tip of the vast iceberg in its understanding of internal immune modulation.\textsuperscript{34} An immune response mounted against internal bodily components was first noted by Dr. William Coley in 1968 when he found that macrophages could mount a response to carcinogenesis.\textsuperscript{35} This proved a notable discovery, opening a range of unknown possibilities for the body’s defense system. For centuries, physicians had understood the concept of bodily defense against external insults (albeit poorly). Finding of modulatory defense mechanisms against internal threats radically altered medicine’s approach to treating illness. In fact, Dr. Coley’s finding brought about a substantial paradigm shift, turning investigative research to consider internal mechanisms that stimulated pathogenesis. As Susan Sontag describes in her treatise “Illness as Metaphor,” pinpointing an internal origin of disease drastically changed patient self-perception. Rather than being viewed as a foreign entity, illness suddenly assumes an esoteric and mystical indwelling as part of the host organism, so that fighting illness is a struggle waged against one-self. Coley’s discovery was roughly concurrent with Nixon’s war on cancer—a federally-legislated struggle against an internal pathology—and the innovative campaigns of Mary Lasker in her own political struggle against cancer.\textsuperscript{36} These generously funded campaigns, however, did not live up to their potential because their magnified intensity was severely limited in scope. Since then, research has broadened its viewpoint in most aspects, approaching cancer as a multifaceted disease or even many diseases confined within a shared category. Today’s cancer research even works in tandem with the human genome project to “map” out a cancer cell’s genome, and research is currently investigating the precise layout of the basal cell carcinoma genome.\textsuperscript{6} These systematic, top-down approaches will offer a more comprehensive understanding of disease while simultaneously noting unique “fingerprint” aspects of cancers that are individually unique. Rather than a monogenic disease, cancer is a body in cellular disarray where cells are nonresponsive to their normal signaling components or are overly stimulated to excessive growth.\textsuperscript{37} In carcinogenesis, normal protective functions
involving cell division have been damaged or downregulated through dynamic molecular factors. Based on this approach, similar tactics are being adopted for understanding neurodegenerative diseases. The seminal observation that internal disarray of molecular messengers and signaling factors is responsible for cancer is a prime corollary for AD. Although all AD patients demonstrate characteristic features of the disease state, many unique factors also emerge. This key aspect underlies the failure behind current drug regimens, which typically treat external symptoms rather than addressing the root molecular cause.\(^{38}\)

**Phospholipase A\(_2\) & Lipid Metabolism**

Of all biological molecules, lipids are adept as energy-rich storage molecules, forming lipid bilayers and micelles of cells, and are involved in many biochemical cascades within the body.\(^{39}\) In fact, lipids are the body’s main energy source and (mole for mole) pack more energy than their polysaccharide counterparts.\(^{40}\) However, the role of lipids as mere storage molecules is growing rapidly antiquated due to recent research. Lipids are critically important to overall health and wellbeing and that lipid composition alone (both in cell membranes and plasma free fatty acids) is vital for health. In fact, researchers have found that free fatty acids (FFAs) and their metabolites can even act as secondary messengers to impact countless molecular interactions.\(^{41}\) Pathways involving the normal metabolism of fats include the AA cascade and the ceramide pathway (sphingomyelinase cascade).\(^{27}\) Taken together, these biochemical pathways are essential for normal homeostatic conditions and are critically important in producing a wide variety of signaling molecules. Imbalances in these cascades, however, can be detrimental and even fatal in some cases. These two major cascades (the AA cascade and the ceramide pathway) display frequent abnormalities in Alzheimer’s disease and other neurological conditions.\(^{42}\) The arachidonic acid (AA) cascade is a highly complex molecular signaling pathway that mediates a wide variety of bodily functions, using lipids as its primary stimulation. The ceramide (or sphingosine) pathway involves the use of COX and LOX enzymes for lipid metabolism.\(^{10}\) Thus, a more thorough investigation of these pathways and their involvement in neuropathogenesis is crucially important for
Phospholipase A$_2$ (PLA$_2$) is a critically important enzyme for the regulation of lipid types within the body.\textsuperscript{43} Occurring in virtually all organisms, PLA$_2$ is a ubiquitous enzyme that exists in six major types: Secreted (sPLA$_2$), cytosolic (Ca$^{2+}$-dependent), calcium-independent, platelet activating factor acetylhydrolase (Groups VII and VIII), lysosomal PLA$_2$, and adipose-specific PLA$_2$ (AdPLA$_2$).\textsuperscript{44} Members of the PLA$_2$ family are organized according to disulfide bonding trends and their order of discovery.\textsuperscript{43} With greater refinement of crystallography techniques in the 1970s, researchers made further inroads into the precise molecular structure of sPLA$_2$. Utilizing X-ray crystallography, researchers uncovered an uncommonly high amount of cysteine residues in PLA$_2$ (over 10\% of its total amino acids), all of which contribute in disulfide bonding.\textsuperscript{45} Collectively, PLA$_2$ enzymes play vitally important roles throughout the body. Through modulating lipid composition of phospholipid bilayers, controlling protein expression, and making critically vital molecules for the body, PLA$_2$ is intimately involved in a variety of bodily functions.\textsuperscript{46} Recent research investigating lipid-signaling cascades has highlighted that tight regulation of PLA$_2$ is essential for proper bodily functions.\textsuperscript{21} This is accomplished through chemical modification (phosphorylation) by MAPK (mitogen-activated protein kinase) at Serine-505.\textsuperscript{45,47,48} Likewise, the presence of high levels of calcium ions (acting simultaneously with phosphorylation of chemical groups) is thought to activate PLA$_2$’s enzymatic activity.\textsuperscript{46,21} The overall three-dimensional structure of sPLA$_2$ is shown in \textit{Figure 1}. PLA$_2$’s modulatory activity in the AA cascade is especially important for preventing neurodegeneration and the creation of pro-inflammatory eicosanoid molecules. The overall molecular structure of sPLA$_2$ is shown in \textit{Figure 1}. Observe the N-terminal (positioned near the lower right corner) and the HI, H2, and H3 $\alpha$-helices (shown as long red coils).\textsuperscript{43} The $\beta$-wing (strangely absent in some plant species) is depicted by antiparallel yellow arrows. The calcium-binding loop (shown in green) is essential in binding and catalyzing substrate.\textsuperscript{22} Notice that sPLA$_2$ consists of only one polypeptide. The C-terminus is located near the top left of the diagram. Seven disulfide bonds are shown in tan, which lend stability to PLA$_2$’s overall structure. The two helical
short turns (SH4 and SH5) are depicted as tight red loops. Interestingly, most aspects of PLA₂’s secondary structure (α helices and beta sheets) are conserved from one species to another. This molecular homology underscores the extreme structural and functional importance of PLA₂’s secondary and tertiary configurations.

![Figure 1: The three-dimensional structure of sPLA₂. Notice that the α helices (shown in red) and the β sheets (shown in yellow) are vital to the protein’s overall conformation. As shown, the cysteine residues are vital to the protein’s function. Each cysteine contains a thiol group, which is adept at bonding with other molecules and/or protonating in solution. Additionally, thiol groups possess the ability to form disulfide bridges—a special biological function.](image)

Arachidonic acid (AA) is a polyunsaturated fatty acid (PUFA) exhibiting a long, 20-carbon skeleton with four cis double bonds (shown below in Figure 2). It cannot be synthesized de novo in humans so it must be obtained exogenously from meats, eggs, and other animal products, or synthesized from its precursor, linoleic acid. Under normal circumstances, the body produces α-linolenic acid. Although some conversion does occur to DHA, it does not occur readily. Therefore DHA is categorized as an essential fatty
acid that must be obtained through diet. Fish and certain plant materials like coconut and olive oils supply \( n\)-3 PUFAs.\(^{50}\) Researchers believe that DHA can modulate gene expression, inhibit inflammation and oxidation, and correctly modulate membrane dynamics.\(^{49}\)

AA is a fatty acid acyl chain with four \( cis \) double bonds. As one of the most common fatty acids in the brain, AA has been linked to spatial learning, image recognition, memory, and critical thinking skills. A study conducted Birch \textit{et al.} demonstrated that infants (18-months) receiving doses of AA demonstrated more rapid brain development compared to controls.\(^{50}\) AA’s role in cognitive development became especially apparent in this study, as infants taking AA supplementation exhibited higher thinking abilities than other infants of the same age.\(^{50}\) When docosahexaneoic acid (DHA) was provided to supplement AA, learning capabilities were accelerated even more dramatically.\(^{50}\) PLA\(_2\), Acetyl-CoA, and COX-2 mediators are all involved in the metabolism and processing of AA and DHA. AA can be produced by linoleic acid (18:2\( n \)-6), but the chemical reaction proceeds slowly in the brain although somewhat faster in the liver.\(^{51}\) These molecules are involved in a series of interrelated pathways all linked to a wide variety of biochemical activity.
As described previously, AA can be obtained exogenously through diet or through chemical modulation of linoleic acid (which is also an omega-6 fatty acid). This occurs through desaturation (addition of double bonds) and subsequent elongation of linoleic acid’s hydrocarbon chain. Once produced, esterification then occurs to incorporate AA into a plasma membrane phospholipid. Along with other 20-carbon fats like EPA and DGLA, AA can form the hydrophobic tail of the phospholipid molecule, which remains buried in the lipophilic portion of the membrane. Recently, researchers have discovered that AA cascade is closely involved in inflammatory activity. When pro-inflammatory environmental factors (exogenous) or endogenous factors are present, phospholipase is stimulated to cleave the bond joining AA with the phospholipid. This liberates AA, leaving it free for oxygenation to occur. Frequently, oxygenation of FFA is carried out by cyclooxygenase (COX) molecules, which cleave two of FA’s alkene groups to add oxygen atoms and increase overall saturation of the molecule. Collectively, these changes produce a wide class of pro-inflammatory molecules called eicosanoids, which are used as secondary messengers that signal inflammation and swelling within the body. As Figure 3 depicts, COX enzymes produce thromboxanes (TX), prostaglandins (PGs), and prostacyclins (PGIs). Since two of AA’s four double bonds have been cleaved, eicosanoids produced in this way contain two double bonds and are classified as “series-2” molecules. Notice that promiscuous enzymes called synthases are used in the creation of new compounds. Cytochrome P450 is a heme-containing protein contained within the mitochondrial inner membrane. It’s activity is crucial for the creation of
stimulatory hormones, cholesterol and vitamin D production, and modification of bilirubin in the liver. 

**Figure 3:** Production of pro-inflammatory molecules from lipid cleavage by PLA$_2$. As shown, epoxides are created through the enzymatic activity of CytP$_{450}$ on AA. COX enzymes produce PGs and TXs, which are present in various forms.

Lipooxigenase (LOX), on the other hand, does not tamper with the alkene bonds and merely oxygenates the FA molecule, ultimately producing leukotrienes (LTs). LTs are intimately involved in the 5-lipoxygenase (5LO) pathway, which activates LTC$_4$, LTD$_4$, and LTE$_4$ (all three are classes of LTs that use cysteine residues’ thiol groups for their enzymatic activity). Further studies have confirmed LTC$_4$’s involvement in weakening of memory and learning abilities. In one study using murine models, neurons were systematically exposed to Abeta$_{1-42}$ oligopeptides (10 μM concentration). This was found to stimulate cysteinyll LTR expression in a concentration-dependent manner (shown below in Figure 4).
normal conditions, AA can exert negative genomic control on its own production. When control of molecular messengers runs amok, however, AA often demonstrates positive control to drive production of LTs and upregulate LOX and COX enzymes.

**Discussion**

Collectively, these data suggest that methods of modulating the AA cascade are essential for AD therapies. PLA₂-mediated cleavage of membrane phospholipids is an important part of lipid metabolism that must occur for regular homeostasis. It appears that lipase activity is not only involved in controlling membrane lipid composition, but also modulating inflammatory factors involved in the immune response. TXs (originally isolated from thrombocytes) and LTs (initially found in white blood cells) are key components of the immunity and cell-cell signaling. This finding suggests that AD
therapies must specifically modulate these signaling cascades by allowing a homeostatic amount of lipase-mediated cleavage to occur. The precise amount of PLA₂ needed is difficult to determine since it varies between individuals and is largely contingent upon diet. As evident from the data presented in this section, a total paradigm shift is needed to bring about substantially persistent results for AD patients. Future therapies must delve to the root cause of AD by addressing the origins and subsequent development of the disease. Despite exhaustive research, a foolproof medication has not been developed. It is clear that abnormal PLA₂ cleavage is a pivotal event for AD development and that the rate of cleavage is essential for determining the fine balance between normal health and AD development. Despite all the collective knowledge about AD, researchers are still uncertain about its precise cause. Its effects (SPs and NFTs) can be easily noticed, but the precise factors driving this disease still remain a mystery. There is a temptation among researchers to develop therapies that target specific portions of the disease phenotype. This position is untenable, however, since AD is a multifactorial illness, causing a plethora of unbalanced activities within the body. Thus, it becomes evident that a therapy that strongly impacts pathogenesis at a very early stage of illness is paramount. Precisely what this therapy entails will be discussed in later chapters.

Chapter 2

Introduction

In addition to the AA cascade, a number of other mechanisms contribute to AD ontology. The major players in this disease include overregulated secondary messengers (such as PGs, TXs, and LTs), a specific kinase called glycogen synthase kinase-3β (GSK-3β), and iron atoms that assault neural tissue. All these molecular factors are implicated in stimulating aberrant secretase activity, upregulating signaling cascades and extracellular plaque deposition, and accommodating neural toxicity. Originally named for its ability to regulate glycogen synthase (a key enzyme in gluconeogenesis), GSK-3β is a kinase enzyme present as two different forms (α and β) that has been implicated in countless bodily processes. Under normal circumstances, it controls energy distribution,
glycogen formation, and establishing the overall body plan during fetal development.\textsuperscript{61} Interestingly, GSK-3β is also involved in neuronal differentiation and its dysfunction is strongly linked to down syndrome (DS) and Parkinson’s disease (PD).\textsuperscript{2} Researchers testing pre-neuronal cells in murine DS models and DS human embryos found that GSK-3β function was increased due to lowered phosphorylation of the enzyme.\textsuperscript{2} By exhibiting a third copy of the AICD portion of the APP gene, DS leads to significant problems with enzyme expression.\textsuperscript{59} Administration of lithium, however, was found to increase phosphorylation to normal levels. This finding may lend credence to the role of lithium therapy in treating AD. This is discussed in greater detail in “Lithium: A Curative Agent?” In this chapter, GSK-3β’s involvement in AD will be examined as well as the varied roles of secondary messengers and the neurotoxic effects of iron in driving AD progression. The role of lithium as a therapeutic will be evaluated and determined in chapter 3.

**Secondary Messengers, GSK-3β, and the Role of Iron**

AA, PGs, and LTs are all secondary messengers that relay chemical signals throughout the body. Dysfunctions involving AA have been linked to many pathologies and chemical reactions that involve PUFAs. Recent research suggests that PUFAs like AA and DHA are deeply involved in membrane dynamics as well as influencing the enzyme conformation and activity, regulating protein expression, and produce vitally important products for use in the cellular environment. These fats are called “essential” because they cannot be produced \textit{de novo}, but rather must be obtained from an exogenous source.\textsuperscript{53} Of all body organs, the brain has an exceptionally large amount of long-chain PUFAs (LCPUFAs). Collectively, AA and DHA occupy over 90\% of fats in the brain, occupying over half of its non-aqueous mass.\textsuperscript{62} In the initial stages of this cascade, PLA\textsubscript{2} is activated to cut phospholipids in the sn-2 position, liberating AA. PLA\textsubscript{2} is upregulated when G-protein coupled receptors (GPCRs) are occupied or when NMDA receptors are activated by glutamate to allow for entry of calcium.\textsuperscript{28} In a widely used, generic form of signal transduction, G-proteins activate adenylyl cyclase (AC), which drives cAMP to interact with protein kinase A (PKA). Once activated, PKA is able to phosphorylate
nearby enzymes to upregulate their activity. Once released, AA can either be reincorporated back into plasma membranes or can undergo further reactions to form a wide class of eicosanoid molecules. These include prostaglandins and thromboxanes (via COX-2); epoxides (via cytochrome P₄₅₀); leukotrienes (via lipoxygenases); or hydroperoxy acids (via autooxidation). Although the precise method of conversion is poorly understood, researchers found that AA can also be converted to anandamide. Collectively, the production of LTs and COX lead to underexpression of DHA, which is important for brain vitality. In fact, multiple studies suggest that DHA may prevent oxidation. Although several trials have shown that supplemental DHA seemed to encourage oxidation in vitro instead of impeding it, most other research shows DHA’s positive effect in counteracting inflammation. DHA may encourage oxidation in some organisms due to its high levels of unsaturation, but in humans most studies have shown beneficial results. Since DHA is useful in treating asthma, researchers speculate that it might favorably impact the cPLA₂ cascade and act to inhibit inflammation. Several in vitro studies revealed that AA is still produced even in the presence of DHA, suggesting that it is not effective in halting the cPLA₂ signaling pathway. DHA and eicosapentaenoic acid (EPA; n-3 C₂₀:₅) both stimulate production of docosatrienes and resolvins, which both aid in neural function. Most notably, DHA is converted to neuroprotectin D₁ (whose production can also occur via sAPPα).

In addition to PKA, another kinase called GSK-3β exhibits regulatory activity through excessive phosphorylation of presenilin-1. Researchers found abnormally increased levels of this kinase within AD brains. Through its enzymatic control of presenilin-1, it is closely linked to secretase cleavage of APP and the production of Aβ oligopeptides. This enzyme plays a key role in the insulin signal transduction pathway and AD symptoms develop when this system runs awry. In fact, recent research suggests an increasingly close connection between type II diabetes and AD. A down-regulated response to insulin is associated with biochemical changes in the phosphatidylinositol-3 (PI3) kinase pathway. Neural tissue carries receptors for insulin and these are regulated through the PI3 kinase pathway and by mitogen-activated protein (MAP) kinase pathway. These pathways are highly dynamic and are chemically regulated on a microsecond basis. Researchers found, for instance, that Akt/PKB
activates the glucose transporter (GLUT) 4 to change its location on the neurolemma plasma membranes. Akt/PKB also negatively impacts GSK-3 through the addition of phosphate groups at the serine 9 position. Interestingly, insulin receptors (IRs) in neural tissue are not only involved in meeting the brain’s need for sugar, but are also closely involved with memory and understanding new concepts. The rate of APP cleavage and tau phosphorylation is closely modulated by IRs. Collectively, these data indicate a closer correlation between type II diabetes and AD than was previously assumed. Glucose uptake by neuronal cells is critically important: on average, the brain consumes an estimated 80% of ingested glucose. Finding efficient methods of controlling the MAP and PI3 kinase signaling pathways is critically important in finding a cure for AD, since it may also impact regulation of secretase activity. Results are currently conflicted regarding concentrations of PI3 kinase: some AD brains demonstrate excessive PI3, while others show a deficit of PI3 kinase. Disruption of these pathways causes increased lactate production and lower ATP production because of disrupted glycolytic pathways. In one significant study, steptozotocin (STZ) was administered in sufficient amounts to cause onset of diabetes mellitus in murine test subjects. Interestingly, mice administered STZ developed disruptions in short-term memory (STM) and several behavioral/social alterations as well. These findings highlight the critical roles of insulin and IRs for neural health, deepening a possible connection between diabetes and AD. These data show that IRs’ collective response to glucose impacts the nervous system tremendously. Further research into neural IRs and their role in STM is essential for discovering the connection between diabetes and AD.

Iron is another factor suspected in the ontology of AD. When APP is transcribed to form mRNA, it can then be translated into an amino acid sequence. When iron is present in large amounts in the brain, it over stimulates translation of the mRNA through iron-responsive factors that are especially sensitive to iron stimulation. Therapeutics are desperately needed to address the potentially harmful role of excess iron in CSF and surrounding neural tissue. Multiple studies demonstrate that iron accumulation occurs rapidly in neural tissue and can encourage Aβ plaque production. Iron and calcium levels within the cell were increased when APP was overexpressed. The precise molecular details behind this process still remain unknown. It is suspected, however, that metals
already present in the extracellular environment are ingested and interact with APP. Researchers also discovered lowered superoxide dismutase (SOD) concentrations in cells with disregulated APP. This is probably due to the overexpression of metal-transporting proteins. Contrary to expectations, transferrin was found to play a minimal role in this process. Iron controls APP functions within the cell. It shares many features for how iron response element (IRE) regulates the translation of ferritin L- and H mRNAs using its 5’ UTRs.

**Sphingomyelinases, the Ceramide Pathway, & Soluble Aβ--**

**Pathogenesis of Inflammation**

Ceramides are a class of fatty compounds made of sphingosine and a fatty acid. Present in abundant amounts within the phospholipid bilayer, recent evidence has found that ceramides are not merely building components of cells but also participate in a wide variety of cellular activities. This activity ranges from differentiation, division, and apoptosis. Ceramides (literally “waxy amides”) are created through several different pathways, one of which is through enzymatic (SMase) breakdown of sphingomyelin. Interestingly, ceramide production upregulates SOCS3, a gene that suppresses kinase signaling. This leads directly to leptin and insulin insensitivity, further establishing the diabetes-AD correlation. Low oxygen, reactive oxygen species (ROS), and UV radiation are all factors that increase SMase activity, leading to the creation of ceramides. When neural tissue is lacking sufficient oxygen, excessive ceramide production was noticed in astrocytes of the hippocampus. This is most likely a response to swelling caused by ischemic conditions.

nSMases are mainly located in the phospholipid bilayer, while aSMases are found in endosomal-lysosomal portions of the cell. Researchers hypothesize that nSMases (especially nSMase2) are involved in AD pathogenesis. nSMase consists of a phosphate group covalently bonded to a protein that contains five serine residues (S173, S208, S289, S292, S299) that are remarkably consistent between species. In the presence of certain stress stimuli, these residues can be phosphorylated to activate the protein. Tumor necrosis factor (TNF)-α, IL-1beta, and IL-6 are all secondary messengers
implicated in stimulated ceramide production. The AA cascade joins the ceramide pathway (which is highly activated in AD patients) through the interaction of these ILs. Researchers noticed major changes in sphingolipid pathways of patients displaying neurodegenerative disorders. Plasma levels of these enzymes were determined by increased levels of SMase and acid ceramidase, resulting in higher ceramide and sphingosine concentrations. Both of these are secondary messengers are intimately involved in cell maturation and death. Both ceramide and sphingosine, for instance, can activate caspases 3 and 9 that trigger apoptosis. It is thought that Aβ upregulates neutral and acid SMases via cPLA₂ and AA. Both kinds of SMases provide structural support for BAC-1, which cuts the β-site of APP. Lowered amounts of SPs in Alzheimer’s patients have been shown to prevent Aβ activity, producing a beneficial neurological effect.

Under normal conditions, AA regulates sphingomyelinase (SMase) function. In AD pathology, however, Aβ peptides activate SMases (both acidic and neutral forms) to induce neural apoptosis. Likewise, antioxidants inhibit SMase by blocking the activity of cPLA₂ or by targeting specific genes using antisense oligonucleotides. Sphingosine-1-phosphate protects the brain by preventing stimulation of acidic sphingomyelinase. Many researchers propose that AD begins with small modifications to the hippocampus as insoluble plaques build up in the extracellular environment. This causes problems with cell-cell signaling through synapses, ultimately causing neuronal death. This theory falls short, however, when one realizes that hardly any correlation exists between dementia and plaque formation. Also, recent research suggests that neuronal apoptosis is linked to soluble concentrations of Aβ peptide oligomers, rather than the fibrous, insoluble kind. Using sections of mouse cerebrum, researchers determined that soluble Aβ oligomers are toxic to the CA1 region of the hippocampus. Several additional studies created transgenic murine models lacking plaque accumulation. Even in the absence of neural plaques, these mice still developed neuroathpies and learning problems. Collectively, these studies lend credence to the theory known as the “soluble Aβ” hypothesis. Sphingolipids (ceramides) and their metabolic products are extremely important aspect of this hypothesis. Ceramide is made through two major methods of production. It can either be made through the breakdown of sphingomyelin by neutral sphingomyelinase (N-
SMase) and acidic sphingomyelinase (A-SMase), which degrade sphingomyelin or manufactured through ceramide synthase.\textsuperscript{73} This signal cascade is present in nearly all cells, but ceramide is present in abnormally high concentrations in AD patients and sphingomyelin amounts were low. This suggests that metabolism of sphingolipids and production of ceramide leads to AD progression. Soluble Aβ leads to cell death because of the cPLA\textsubscript{2} pathway that makes AA. Also, excessive oxidation of fats, nucleic acids, and proteins has been linked to damage caused by soluble and fibrous Aβ peptides.\textsuperscript{24,26}

In one significant study, immunoblot analysis and SDS-PAGE were used to determine the histological location of Aβ peptides \textit{in vitro}. After this, MAFP (methyl arachidonyl fluorophosphate) was used to inhibit cPLA\textsubscript{2}. By stopping the activity of cPLA\textsubscript{2}, AA liberation was prohibited. Through this study, the researchers found that excessive production of AA is harmful to neurons of the cortex by stimulating SMase activity. By using an MTT assay, the study found an inverse relationship between arachidonic acid concentration and neuronal viability.\textsuperscript{13} Also, neurons exposed to AA longer suffered more toxic effects. Neurons exposed to 5 µM of AA, for instance, demonstrated an upregulation of N- and A-SMase activities even after only one hour of incubation.\textsuperscript{80} Maximum SMase production occurred after 6 hours of exposure. Desipramine and 3-O-methyl-sphingomyelin (3OMeSM) inhibit SMases and these stopped apoptosis caused by AA. Results were also tested using different fatty acids others than AA (stearic acid, oleic acid, and linoleic acid). Interestingly, the introduction of these fatty acids did not lead to apoptosis or upregulation of SMase activity. These results are supported by a similar study (Florent \textit{et al.}) showing that docosahexaenoic and eicosapentaenoic acids did not induce apoptotic activity in neurons.\textsuperscript{49} In fact, the results suggested that these additional fatty acids were helpful for neuronal activity, while Aβ oligomers may induce A- and N-SMase production. Antisense oligomers, Aβ (40-1), were used as a control and did not induce SMase production. 3OMeSM and N-acetyl cysteine (NAC) inhibit SMase and helped preserve neuronal integrity. Desipramine and imipramine both inhibit A-SMase and also lead to lower rates of cell death. Strangely, however, 3OMeSM and desipramine both encouraged cell death.\textsuperscript{73} This finding puzzled researchers and still cannot be properly explained. Fumosin B1 inhibits ceramide synthesis and inhibition of serine-palmitoyl transferase (a gateway enzyme for creating
sphingolipids) caused no changes in rates of cell death. From this data, researchers concluded that prevention of SMase activity greatly benefitted neuronal function by lowering caspase-3 and caspase-9 activity (caused by soluble Aβ peptides).\textsuperscript{75} Amazingly, 1 µM concentrations of soluble Aβ peptides caused a 51% lowering of cell livelihood after 24 hours of incubation. 3OMeSM and desipramine lowered soluble Aβ1-42-induced apoptosis to 75 and 81%. Also, a study found that ceramide utilizes cathepsin-D (which is manufactured by lysosomal A-SMase).\textsuperscript{27} When cathepsin-D was inhibited by an octapeptide, this proved highly beneficial to neurons. Thus, overexpression of the ceramide pathway (and associated overproduction of N- and A-SMases) are detrimental to neural health and can stimulate development and progression of AD.

**Genetics & Environmental Risk Factors**

The risk of AD and cerebrovascular disease (CVD) is highly increased with the possession of Apolipoprotein ε4 (APOE4) gene. Strokes are often a common side effect with ApoE4 because CVD usually exhibits a phenotypic impact (like a cerebral infarction) that cannot be noticed clinically. Unfortunately, many cerebral infarctions remain unnoticed and can lead to strongly detrimental effects like stroke.\textsuperscript{81} Interestingly, researchers have correlated white matter hyperintensities (WMH) to stroke incidence. For individuals older than 65-years-old, AD is the fourth leading cause of morbidity. In the initial stages, AD first affects thinking and memory and later muscle control.\textsuperscript{14} Hypertension, diabetes, smoking, high blood homocysteine, and previous strokes are all thought to increase chances of AD onset.\textsuperscript{82} “Late-onset AD” is more common and more closely correlated to APOE4. “Early-onset AD” is correlated to a set of three genes. The first codes for β-amyloid precursor protein (APP) of chromosome 21.\textsuperscript{42} The second codes for presenilin-1 (PS1) on chromosome 14.\textsuperscript{77} The third codes for presenlin-2 (PS2) on chromosome 1, which aids in presenilin degradation. These genes drive upregulation of astrocytes and microglia, ultimately stimulating AD onset. Interestingly, demographic studies have noticed AD’s infrequency in India and other parts of Asia. It is often debated whether this is a result of diet or is genetically linked and/or cased by some other
unnoticed factor. Genomic testing reveals that the ApoE4 gene is extremely low in India. Because of their genetic variability, most Indian AD patients demonstrated differences in disease progression, showing lower levels of oligometric $\text{A}\beta_{1-42}$ peptides than their American counterparts, as well as less heavily phosphorylated tau. These studies still remain unclear and further testing is needed to determine the importance of these studies for current Alzheimer research.

**Impact of Neuroinflammation**

Although tau aggregation and SP deposition are important in disease pathology, neural inflammation seems to be the driving factor. This is hypothesized to occur through excessive stimulation of AA and the production of pro-inflammatory eicosanoids mentioned earlier. In one significant study, researchers used spontaneously hypertensive rats (SHRs), which are characterized by hypertensivity, diabetic symptoms, and other metabolic disorders. SHRs were chose because of their propensity to create eicosanoid molecules, while Wistar-Kyoto (WKY) controls exhibited normal blood pressure and produced fewer inflammatory factors. SHRs were found to demonstrate a more rapid progression of AD, most likely due to widespread microglial activation. Microglial are a special type of phagocytic cell within the nervous system that is stimulated to consume threats to homeostasis. In the presence of inflammatory stimuli, microglia are alerted and produce a variety of cytokines. Unfortunately, in the SHR rats this only lead to further stimulation of tissue swelling. Under inflammatory stimuli, mitogen-activated protein kinase (MAPK) is hyper-activated, leading to increased NFTs. This overall process is shown below in *Figure 5*. 
studies show that NFTs stimulate inflammation, while others suggest that pro-inflammatory cytokines precede disease onset. A similar study (Yoshiyama et al.) also demonstrated these results using tau transgenic (Tg) mice. One of the researchers’ key questions was whether inflammation or neural necrosis is the driving force behind AD. Researchers found that abnormal tau expression may be caused by cytokine

**Figure 5**: Tau aggregation in AD neural tissue. Abnormal tau expression leads to microglial activation, which contribute to neuronal death. Notice that the microglia clump together in response to chemokine signals from neighboring cells.

Results are currently conflicted regarding inflammation’s role in neurodegeneration. Some
overproduction, a phenomenon that Zilka et al. have creatively called “cytokine storm.”\textsuperscript{19} When brain cells are damaged through ROS, for example, they can stimulate over-activation of microglia, which upregulate their phagocytic activity to form large clusters in the nervous system. This triggers an immune system response, which crowds blood plasma with macrophages.\textsuperscript{83} Strangely, researchers found that immune system activation can actually accelerate tau aggregation—ironically stimulating the very process it was striving to prevent. By manipulating the fractalalike-receptor for microglia (CX3CR1) and the ligand for microglia (CX3CL1), researchers hoped to find a method of opposing immune-stimulated neuroinflammation.\textsuperscript{19} Although extracellular levels of SPs decreased in CX3CR1 knockout mice, intracellular tau protein concentrations increased.\textsuperscript{19} Hence, Zilka et al.’s study demonstrated mixed results: inhibition of the receptor lowered insoluble plaques, but lead to an increase in NFTs. Although largely unsure why this occurred, Zilka et al. concluded that their data supports the hypothesis of inflammation-induced AD.\textsuperscript{19,74} This is certainly a matter of debate that cannot be conclusively decided; however, the most current data suggests that neurodegeneration and the inflammatory cascade can be largely attributed to the actions of the esterase PLA\textsubscript{2} and its enzymatic functions in the AA.\textsuperscript{45}

**Discussion**

More than any other single finding, GSK-3β’s effect on neural insulin receptors underscores the relationship between diabetes and AD. The precise molecular factors that link these two illnesses must be researched in greater detail. How caloric (and sugar) intake affects the metabolism of membrane lipids will be especially pertinent to this area of study. Also, cholinergic receptors (typified by a positive response to acetylcholine) are prime targets for future therapies. Finding methods of artificially stimulating these receptors (especially through Galantine) may prove promising and must certainly be studied in greater detail. Any potentially harmful effects of Galantine overstimulation must be researched in greater detail. As a majorly activated cascade in AD, the ceramide pathway must be targeted by a therapy that effectively addresses the disease. In particular, SMases must be brought to normal plasma concentrations and ceramide recruitment of caspase-3 and -9 must be inhibited. Also, although SPs and NFTs are a
major issue, neuroinflammation seems to be a deeper problem. Research seems to indicate that inflammatory signals are the motive driving force behind plaque deposition and NFT accumulation. Therapies that specifically impede internal inflammation by inhibiting enzyme overactivity (and the related “cytokine storm”) are sorely needed. Also, patients who have suffered a stroke must be subjected to preemptive treatments to halt pathogenesis and monitor the development of WHM. The immune-mediated response of microglia is not intrinsically detrimental, but overactivated glia leads to apoptotic activity. Following this theme, chapter 3 will present various current therapies used to address AD symptoms. Finally, the therapeutic efficiency of turmeric will be noted in comparison to other treatments.

Chapter 3

Introduction

Drawing from copious amounts of data, researchers are hoping to form a constructive framework for an increased understanding of AD pathogenesis and development. By direct chemical modulation of the AA and ceramide cascades (and interception of the secondary messengers involved), researchers hope to design effective drug regimens for AD patients. Thus far, the search for a cure has proven only moderately successful. Due to the body’s complex molecular interchanges and formation of intermediary structures, development of effective therapies with minimal side effects is an extremely difficult endeavor. COX-2 inhibitors are a subject of special interest for researchers, since modulation of these oxygenase molecules will prove vitally useful in treating AD. However, as this chapter shows, even the most carefully concocted therapies fall short of their desired intent. This failure is due mainly to ineffective metabolism, misdirected tissue targeting, or through complications caused by adverse effects. As discussed in this chapter, rofecoxib and celecoxib both exhibit therapeutic promise, but are no longer widely used because of their potential fatality. This chapter will examine many common pharmaceutical regimens used for AD patients, their mechanism of action (if known), and their comparative success. Also, the cholinergic
receptor hypothesis will be discussed relative to AD and a brief evaluation of how different drug classes influence brain activity will be presented. Finally, the need to incorporate turmeric into Western medicine will be presented and curcumin’s influence on body systems will be examined in greater detail. Although not exhaustively researched, turmeric’s medicinal benefits can be strongly inferred from numerous studies. These studies indicate that turmeric offers a therapeutic remedy to replace ineffective COX inhibitors, NSAID drugs, and other compounds like memantine.10,88

**Searching for a Cure—Inhibitors and Molecular Intervention**

Current medical treatments recognize the extreme importance of decreasing inflammation, since it causes (or at the very least aggravates) neurodegeneration. Also, treatments that address neurotoxins (such as rising iron concentrations and overexpression of Aβ) are essential for treatment of AD. Current treatments seek to address these issues using a variety of methods. First, there are molecular inhibitors that specifically antagonize the activity of particular enzymes. COX-2 inhibitors are perhaps the most widely known of this category. Cox-2 inhibitors (rofecoxib, celecoxib, and others) have been used therapeutically, but exhibit less than desirable results (in some cases even causing death). Likewise, glucocorticoids like prednisone have demonstrated no observable improvements for memory, learning, and social interactions.89 COX-1 has been shown to cause some inflammatory activity but it is also highly involved in digestion, so its activity cannot be inhibited without causing gastrointestinal problems.19 Although NSAIDs were found to reduce swelling, they were inefficient at reducing Aβ plaques.10 The inefficiency of most pharmaceutical drugs is due to a great deal of educated guesswork and trial-and-error. In many ways, the drug design process itself is experimental, based upon hypothesis and tweaking of the final drug product based upon experimental results.90 Up to this point, these therapies have yielded highly discouraging results. The most efficient therapy would effectively address swelling, reduction of neuritic plaques, downregulation of tau protein, and normal regulation of PLA2. Once these issues are addressed, AD can be effectively controlled. Also, an effective drug
regimen for AD would address genomic regulation, which is often the source of enzyme dysfunction.

**Lithium—A Curative Agent?**

Since the 1950s, lithium has been advanced as a neurological therapeutic for mood and general neural homeostasis. Lithium has a long history of therapeutic use for neurodegenerative diseases. Originally used to treat gout in the 1840’s, lithium has also been clinically administered to bipolar patients for over 50 years. It has also been used as an adjunct for blood pressure medication. The precise molecular mechanism of lithium within the brain is poorly understood and it was prohibited after demonstrating negative side effects, including the deaths of four patients. Many researchers hope it may prove effective in treating neuropathies like AD. Lithium—originating from the Greek “Lithos” (stone) since it was originally found in rocks-- was first discovered by Arfwedson, a Swedish chemist, in 1817. Lithium exists as a positively-charged monovalent atom that is interchangeable with other cations like potassium, sodium, and even divalent atoms like magnesium and calcium. In the laboratory environment, lithium acts catalytically on uric acid in the kidneys, although significant amounts are needed to induce these results *in vivo*. By the 1880s, it was clinically prescribed for chronic depression and other psychological abnormalities. By 1940, physicians realized a correlation between sodium-rich diets and hypertension, and lithium was used to substitute sodium chloride as a treatment for blood pressure. But in 1949, Corcoron *et al.* showed that lithium could cause widespread tissue damage and even death, and its use as a therapeutic was prohibited across America.

This large-scale discrimination against lithium began to fade, however, when a study conducted by Cade noted the sedentary effect of lithium on guinea pigs. Cade theorized that mental problems were caused by a hitherto unrecognized poisonous agent, which was then excreted in urine. In one notable study, Cade injected lithium urate into guinea pigs, causing them to become sedated. Lithium chloride is the only dissolvable salt in uric acid, which lead to sedation. This study reinvigorated the therapeutic use of lithium carbonate in the treatment of neurological diseases. Interestingly, lithium
carbonate showed no effect in schizophrenia or depression, but it was effective in all ten manic patients in one study.\textsuperscript{93} After these hopeful results, lithium chloride became the first FDA-approved drug for bipolar disorder.\textsuperscript{96} The practice of prescribing lithium may have historical precedent. In the 5\textsuperscript{th} century A.D., Caelius Aurelianus recommended alkaline water for mentally disturbed patients, which may have contained high concentrations of dissolved lithium ions.\textsuperscript{91} At this point, research remains inconclusive and further studies must be done to determine the precise mechanisms of lithium’s clinical efficacy. However, recent studies have uncovered the chemical interactions involved in lithium’s usefulness as a therapeutic. Recently, lithium has been shown to impact adenylyl cyclase (AC), which is an important cellular modulator for countless chemical reactions.\textsuperscript{61} Both cyclic adenosine monophosphate (cAMP) and AC modulate the effects of cAMP in the body for countless biological reactions. AC transforms ATP to cAMP by way of a multistep process. Then cyclic nucleotide phosphodiesterase changes cAMP to AMP.\textsuperscript{95} This is a common event in signal transduction pathways, followed by subsequent phosphorylation of specific protein targets. Protein kinase A (PKA) is involved in this process through the removal of phosphate molecules.\textsuperscript{91} Lithium upregulates cAMP and PKA activities by increasing enzyme binding efficiencies. Thus, lithium serves as a metal catalyst that improves reaction conditions and accelerates the reaction. Mori \textit{et al.} found that lithium prohibits the transfer of phosphate groups by PKA in microtubule sections of rat brains. This is accomplished when lithium vies with magnesium at the C subunit of PKA.

Recent research suggests that lithium may play crucial roles in protecting neurons and may be therapeutically valuable in treating AD, Parkinson’s, Huntington’s disease, Amyotrophic lateral sclerosis, and ischemic conditions. Also, it can block N-methyl-D-aspartate (NMDA) receptors and helps neuroregulatory proteins. As one of the first proteins discovered that regulates apoptotic activity, B-cell lymphoma/leukemia-2 (Bcl-2) gene can antagonize potential hazards like absence of growth factors, radiation, glucocorticoid hormones, and oxidative agents like hydrogen peroxide.\textsuperscript{97} Bcl-2 upregulates caspase and increases mitochondrial calcium influx. Lithium causes higher Bcl-2 levels in the cortex, hippocampus, and striatum and also lowers concentrations of protein 53, which normally induces apoptosis.\textsuperscript{97} It also stimulates Akt, a class of protein
kinases that specifically utilizes serine and threonine and is controlled by the phosphatidylinositol kinase molecular cascade. GSK-3β prevents overproduction of β-catenin by degrading it over time. Long-term administration of lithium increases β-catenin in murine models. AD is frequently associated with misreadings in presenilin protein, which leads to lower levels of β-catenin. This is linked to apoptosis by Aβ plaque accumulation. Presenilin-1 joins with β-catenin, leading to a lower energy state. Low levels of GSK-3α are directly correlated to Aβ concentrations, whereas GSK-3β produces the inverse effect. Prevents Aβ being made from APP. A study by Feyt et al., however, offers conflicting data to these studies involving lithium as a therapeutic agent for AD. Thus, although lithium should be considered a viable candidate in treating AD, much more research is needed to prevent detrimental effects. Lithium may be incorporated as a therapeutic, but only after plentiful research has been conducted to verify its beneficial effects while preventing neurotoxicity.

**New Frontiers-- Memantine & NSAIDS for AD Therapy**

Cholinergic neurons are characterized by their responsiveness to acetylcholine and upregulating cholinergic neuron activity is a prime target for therapies. Molecules that antagonize cholinesterases are the most efficient type of inhibitor used because they prevent the enzymatic deconstruction of cholinergic receptors. Through conservation of these receptors, acetylcholine is encouraged to remain within the synaptic cleft and participate in vital neural functions. Galantamine, Rivastigmine, Donepezil, and Tacrine are four of the most commonly used compounds to prevent cholinesterase activity. Drugs that exhibit this type of activity are currently the only officiated drug for AD. As AD progresses, however, these treatments grow less potent, gradually losing their efficiency over time. It is hypothesized that cholinesterases gradually lose their responsiveness to treatments or find methods of avoiding the intended therapy. Thus, although cholinesterase inhibitors are headed in the right direction, they are not a viable, long-term solution. NMDA inhibitors are also a topic of interest. Memantine, in particular, is especially effective through its prohibition of excessive AA release. The process of synthetic memantine production is shown below in Figure 6. Although memantine may
Nonsteroidal anti-inflammatory drugs (NSAIDs) are compounds meant to counteract AD pathology. Aspirin, the most commonly used NSAID, is now prescribed for a variety of ailments. Now it is often taken to preemptively stop the occurrence of strokes and myocardial infarctions. As the most commonly utilized drug in Western medicine, aspirin is useful for a variety of activities, including reducing fever, pain, and inflammation. Multiple studies suggest that aspirin taken in combination with DHA may prevent the pro-inflammatory activity of AD. When placed in the same chemical environment as omega-3 PUFAs, aspirin leads to the production of resolvins, protectins, and oxoderivatives that collectively protect neurological tissues.

AD is often associated with vascular dementia (VaD), which is typified by abnormal, undetectable growth of the brain’s capillaries. This occurs early on in the disease and often remains undiscovered clinically. In fact, VaD phenotype is so internalized that autopsies are the most frequent method of VaD detection. Elderly patients often display inflammation as a normal sign of aging; unchecked, however, inflammation can quickly turn pathological over time. As plasma cytokine concentrations rise, astrocytes...
swell in a phenomenon known as astrogliosis.\textsuperscript{74} Found in the central nervous system (CNS), astrocytes are star-shaped cells that participate in a wide variety of reactions, such as providing structural support and nourishment to neurons. They also constitute the lining of the blood-brain barrier (BBB), exchange substances between neural parenchyma and blood plasma, communicate through surface contact with other neurons, increase synapse connections, and facilitate signal transduction from one neuron to another.\textsuperscript{75} Astrogliosis stimulates a vicious cycle where inflamed tissues release more pro-inflammatory molecules, leading to deadly results.

Receptors in the brain are divided into two major categories: nicotinic and muscarinic. Nicotinic receptors are typified by ligand-gated ion channels embedded in the neurolemma and at the neuromuscular junction. Both receptor types are cholinergic, meaning that they respond to acetylcholine to propagate a signal along the axon to a neighboring neuron, or to directly stimulate muscle contraction.\textsuperscript{98} Although both are responsive to ACh, they are differentiated by their response to other compounds. For instance, nicotinic acetylcholine receptors (nAChRs) are characterized by their response to nicotine, while muscarinic acetylcholine receptors (mAChRs) are stimulated by muscarine-- a substance originally found in the poisonous mushroom \textit{Amanita muscaria}.\textsuperscript{98} mAChRs are G-protein coupled receptors (GPCRs), which is the starting point for the types of signal transduction described earlier. nAChRs are severely depleted in AD. As discussed previously, cholinesterase inhibitors (ChEIs) are frequently used during early stages of the disease, but they do not provide optimal results and show limited efficiency. Researchers suspect that poor response may be due to gene variability. Single nucleotide repeats (SNRs) were noted in \textit{PRKCE} and \textit{NBEA}, which are both gene regions associated with cholinergic receptor functionality.\textsuperscript{101} α-nAChR is mainly conserved between individuals and plays key roles throughout the CNS, originating from \textit{CHRNA7} (on chromosome 15q14).\textsuperscript{101} Researchers noted that genetic differences in this gene can impede AD development, slowing down disease progression in some patients. Interestingly, gender differences can contribute genetic variations for how these receptors are encoded and expressed. More than likely, this is due to the influence of sex hormones
Control of nAChR by Galantamine (a ChEI) is depicted below in Figure 7.

Many studies noted the connection that many nonsteroidal drugs to prevent arthritic inflammation demonstrated lower AD incidence. Likewise, multiple animal studies noticed that Aβ and tau protein concentrations decreased upon administration of these nonsteroidal drugs. Although the underlying mechanism for these drugs’ efficiency is still unknown, it is suspected that γ-secretase is antagonized by these drugs. Inhibition of γ-
secretase would prevent cleavage of APP (and subsequent production of Aβ oligopeptides).22

Although some progress has been made in recent years, a definitive treatment that is viable for AD has not been proposed. Current treatments (such as NSAIDS and COX-2 inhibitors) are merely palliative, dealing only with disease symptoms rather than addressing the root cause. Also, patient variability is another factor to be considered: individuals respond to drugs differently because of various environmental and genomic factors. Because molecular conditions vary so drastically between individuals, there is no cure-all treatment for AD, just as there is no “silver bullet” to defeat cancer. If Alzheimer’s is to be cured, a drug (or drug regimen) must be found to deal with the symptoms while also attacking the root cause of the disease. Current medicinal methods of treatment (although possibly delaying AD onset) do nothing to prevent neural damage and cannot ultimately save the patient. Against the background of failed medicinal treatments, turmeric emerges as a novel therapeutic compound that may prove highly efficient in preventing and permanently curing AD. The therapeutic values of turmeric as well as common critiques against its efficiency will now be evaluated in greater detail.

**Turmeric Absorption & Bioavailability**

Turmeric is often critiqued for its poor bioavailability levels, especially in elderly test subjects. This is an expected phenomenon: phenolic ring systems and their associated hydrocarbon chains in curcumin are not easily dissolvable in the aqueous plasma solution. Although the hydroxyl and ether groups are water-soluble, these represent a very small portion of the curcumin molecule. Also, research suggests that metabolism of turmeric occurs too quickly to produce healthful effects. Phenolic groups have traditionally been viewed as relatively inactive. Often found in flowers, fruits, nuts, and roots, phenols have been seen as unimportant to overall wellbeing. Recently, however, researchers have found that these groups may play essential roles in stopping cancer, protecting against oxidative damage, and preventing inflammation. In fact, they may prove especially important in brain and liver health. Phenols strongly lend
electrons and form sturdy intermediates due to hyperconjugation of their aromatic systems.\textsuperscript{107} This provides them with a high degree of chemical stability. Some studies suggest that a turmeric regimen supplemented with probiotics may produce enhanced curcumin absorption from the stomach.\textsuperscript{108} When turmeric is obtained and freshly ground, it is often accompanied by lactic acid bacteria (LAB) that aid substantially in its metabolism and eventual digestion. In one study, a ferric-reducing antioxidant power (FRAP) assay was used to determine the degree to turmeric (in the presence of LAB) counteracted ROS.\textsuperscript{3} Rats were given a turmeric-containing solution that was fermented with LAB. These rats demonstrated much higher absorption levels than humans who imbibed turmeric without LAB. This finding suggests that therapeutic turmeric should be as fresh as possible (so it contains LAB), or should be fortified with LAB probiotics.\textsuperscript{109} Also, like most other compounds, turmeric is best absorbed when ingested with a meal. This leads to greater production of bile salts within the small intestine that can help emulsify water-insoluble portions of curcumin.\textsuperscript{110} Certainly, future studies are needed to elucidate optimal methods of turmeric delivery. Also, to date no studies have been conducted regarding optimal dosage of turmeric for mammalian systems. Although many of turmeric’s molecular mechanisms still remain hazy, substantial evidence supports the hypothesis that turmeric will prove an effective therapeutic for AD.

**Curcumin—A Therapeutic Solution**

Turmeric (*Curcuma longa*) is a plant grown in much of southeast Asia. Especially common in India, it is related to the ginger family and its dried rhizomes are usually consumed as a spice.\textsuperscript{111} Turmeric is a yellow-brown color and exhibits a mildly spicy taste. As a diarylheptanoid molecule, curcumin (diferuloylmethane) exists as a substituted heptene chain flanked by two substituted benzene molecules.\textsuperscript{106} Curcumin is categorized as a beta-diketone molecule, which lends special chemical and medicinal activities. These phenolic groups contribute to turmeric’s brown-yellow color. As a racemic molecule, it exists in both keto and enol forms.\textsuperscript{108} The enol form exists in higher concentrations since it is more energetically favorable. In *Curcuma longa*, curcumin’s biochemical production uses phenylalanine as a starting reactant. Turmeric contains over
20 bioactive molecules, of which curcumin occupies approximately 3% of all turmeric’s biomolecules. Circuminoids exist as three major forms: diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin. The first of these, diferuloylmethane, is the circuminoid found in turmeric. Turmeric has been evaluated in many different studies. One study measured the effects of suberoylanilidehydroxamic acid (SAHA) and curcumin used together on Aβ plaques. Used in tandem, they were found to increase learning and memory in transgenic rat models. Also, both these compounds were discovered to protect neurons from the toxic effects of soluble and insoluble plaques. In a separate study, researchers demonstrated that BDMC33 (a modified form of curcumin) prevented the production of nitric oxide among macrophage cells, thus partially inhibiting inflammation among body systems. PGs are common cellular components that are naturally manufactured by nearly every cell type. They are made from AA and serve as important lipid-derived secondary messengers in the body. As a vitally important PG, the E2 variety is chiefly modulated by COX, which is also known as prostaglandin endoperoxide H2 (PGH2) synthase. Current research has uncovered three major COX molecules. Under normal conditions, COX-1 is active without any form of enzymatic stimulation. COX-2, on the other hand, is inducible and is upregulated by the presence of cytokines, cancer-stimulating genes, and various growth factors. Although not thoroughly understood, COX-3 was found to possess intron-1. Researchers found that cellular overexpression of PGE2 and COX-2 lead to a number of diseases, such as asthma, arthritis, and AD—all related to pro-inflammatory activity. In multiple studies, turmeric was found to prevent biological synthesis of PGE2. NSAIDS are designed to specifically block pain caused by inflammatory disorders. COX-1 plays vital roles in protecting the GI tracts, so researchers must be careful that NSAIDS do not block this activity as well. Turmeric therapy solves this problem by effectively inhibiting PGH2 synthase (COX-2) activity, while not interfering with the normal homeostatic role of COX-1. Figure 8 shows the pharmaceutical production of BDMC33. Notice that the basic cyclical structure of curcumin is conserved and that a number of oxygen atoms with lone-pair electrons (in the methoxy and carbonyl groups) are present.
BDMC33 in particular was found to stop nitric oxide synthesis from IFN-γ/LPS macrophages.\textsuperscript{114} Also, it partially inhibited COX-2 and PGE\textsubscript{2} activity. Additionally, it acts against free radical species that can cause major damage within the body. If not for the intermediary activity of curcumin, ROS could damage neural proteins, fats, and genetic sequences.\textsuperscript{111} Left unchecked, free radical species ultimately lead to carcinogenesis or widespread apoptosis. Also because of its unique molecular structure, curcumin is able to “catch” free-floating metal ions, thus preventing them from damaging neural tissue.\textsuperscript{116} Entrapment of metal ions like copper and iron prevents activation of the NF-B pathway, thereby inhibiting production of Aβ plaques.\textsuperscript{78}

In multiple studies, curcumin has also been shown to decrease inflammation through downregulation of eicosanoid molecules, both in neural tissues and in the bloodstream.\textsuperscript{53} In fact, curcumin is also known as an effective blood pressure medication in many Eastern cultures.\textsuperscript{118} Ingestion of turmeric causes greater vasodilation (which produces the same health effect caused by an aerobic workout). In fact, when moderate amounts of turmeric ingestion is paired with aerobic exercise, results are markedly improved.\textsuperscript{103} Thus, the potential benefits of aspirin in preventing inflammation are miniscule compared to curcumin’s potent effects. It is critical that future studies examine turmeric’s effect on swelling and blood pressure in greater detail.
By modulating key molecular players early in the pathway of amyloidogenesis, curcumin therapy cuts straight to the heart of the issue. It precisely targets vital molecular messengers like PGH$_2$ and COX-2, directly impacts excessive enzyme activity by targeting PLA$_2$ and ceramide, and prevents ROS damage. Unless taken in exorbitantly high doses (> 1 kg) intravenously, curcumin poses no known threats to body systems. Excessive ingestion of turmeric merely leads to discoloration of feces and diarrhea without any other known complications. Compared to the side effects of COX-2 inhibitors (which can cause severe indigestion, insomnia, and headaches), the secondary effects of turmeric are virtually nonexistent when taken in normal amounts. Rather than attempting to treat AD symptoms, curcumin delves to the source of the problem by stopping APP’s production of A$\beta$ peptides. It also plays a modulatory role through precise mediation of lipid catabolism pathways. Multiple studies show that curcumin lowers inflammation, disposes of ROS, and prevents accumulation of plaques. It is also suspected to inhibit a wide variety of cancers. Demographics that consume high amounts of turmeric tend to demonstrate lower rates of Alzheimer’s. Although demographics cannot conclusively prove that a curcumin-rich diet is responsible for a healthful phenotype, this correlation cannot be overlooked in light of previous data.

Turmeric demonstrates considerable medicinal activity for treatment of oncogenic activity. In one study, murine macrophage RAW264.7 cells and HT-29 carcinogenic human colon cells were both tested to determine turmeric’s effect on lipid cascade activity and its impact on concentrations of select proteins. Cystolic phospholipase A$_2$ (cPLA$_2$) levels were measured as well as COX and 5-lipoxygenase (5-LOX). First, cells were activated using lipopolysaccharides (LPS), which upregulated COX-2 production. Tetrahydrocurcumin (THC) and curcumin both exerted an inhibitory effect on the AA cycle so that AA was not liberated to produce pro-inflammatory eicosanoid molecules. It seems that this was largely due to prevention of cPLA$_2$ phosphorylation. The enzyme itself was not inhibited; rather, activation through phosphorylation was prevented. This is an important distinction because it means that curcumin did not knock out total enzyme function. Even in the presence of curcumin and THC, cPLA$_2$ was still able to conduct its normal activities, but without excessive phosphorylation. Every curcuminoid tested in this study caused lower levels of prostaglandin E (PGE$_2$). Thus,
curcumin proves itself to be a potent COX inhibitor. It is used in treating many cancer types as well as gastrointestinal problems. It also acts against free radical species that can cause major damage within the body and can protect neurons from environmental toxins and also endogenous factors.

Through the mediatory activity of curcumin, turmeric effectively addresses all the major issues caused by AD—both the internal environment and at the genomic level. It addresses PLA$_2$, reduces the activity of ROS, prevents excessive methylation of key genes, and targets TNF-$\alpha$. Its concise modulatory activity is crucial for terminating A$\beta$ plaques and the dissolution of NFTs—essentially curing Alzheimer’s.
Table 1: The effects of curcumin on mechanisms involved in the degeneration in Alzheimer’s disease

<table>
<thead>
<tr>
<th>Mechanisms involved in degeneration in Alzheimer’s disease</th>
<th>Effects of curcumin</th>
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<tbody>
<tr>
<td><strong>β-amyloid</strong></td>
<td>Decrease in β-amyloid&lt;sup&gt;117&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Increased production</td>
<td>Inhibition of sheet formation&lt;sup&gt;115,116&lt;/sup&gt;</td>
</tr>
<tr>
<td>• β-sheet formation</td>
<td>Decrease neuronal toxicity&lt;sup&gt;107&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Neurotoxicity</td>
<td>Decrease NF-κB activation&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td>• NF-κB activation</td>
<td>Decrease ERK-1/2 expression&lt;sup&gt;109&lt;/sup&gt;</td>
</tr>
<tr>
<td>• ERK-1/2</td>
<td>Inhibit γ-secretase&lt;sup&gt;119&lt;/sup&gt;</td>
</tr>
<tr>
<td>• γ-secretase activity</td>
<td>Modulate presenilin-I&lt;sup&gt;119&lt;/sup&gt;</td>
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<tr>
<td>• Presenilin-I mutation</td>
<td></td>
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<tr>
<td><strong>Oxidative stress</strong></td>
<td>Decrease IL-1β&lt;sup&gt;123&lt;/sup&gt;</td>
</tr>
<tr>
<td>• IL-1β</td>
<td>Decrease GSK-3β&lt;sup&gt;113&lt;/sup&gt;</td>
</tr>
<tr>
<td>• GSK-3β</td>
<td>Prevent β-amyloid induced increase&lt;sup&gt;110&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Caspase-3</td>
<td>Activate neuroprotective pathway&lt;sup&gt;110&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Akt</td>
<td>Iron chelation&lt;sup&gt;120&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Other</td>
<td>Decrease phosphorylation</td>
</tr>
</tbody>
</table>

**Abbreviations:** ERK-1/2, extracellular signal-regulated kinase-1/2; GSK-3β, glycogen synthase kinase-3β; IL-1β, interleukin-1 β; NF-κB, nuclear factor-κB.

Figure 10: Therapeutic roles of turmeric. As outlined in this section, curcumin directly impacts secretase, presenilin, GSK-3β activity and also provides increased neural protection against exogenous metals, tau proteins, and extracellular plaques.
involved in AD ontology and progression. Turmeric offers a safer and more effective treatment than NSAIDS, cholinesterase inhibitors, or anti-inflammatory medications.\textsuperscript{108} Future studies must investigate the role of turmeric paired with other therapeutic compounds, such as liquiritin, CP, and shankhpushpi. To date, several studies have underscored the importance of CP and liquiritin, but none have ever investigated potential synergistic effects when used in tandem.\textsuperscript{119}

**Prospects for Future Studies**

**Study Purpose:** The main purpose of this study is to determine the efficacy of turmeric for the treatment of AD. Hence, the aims of this prospective study are two-fold. First, this study will determine turmeric’s impact on amyloid-β plaque deposition in transgenic AD models in very early stages of the disease. Secondly, it will determine the effect of turmeric on improvement of spatial learning, coordination, and balance in stage 2 Alzheimer mice.

**Experimental Design:** This study will be performed across a span of six months using a total of 100 test subjects (*Mus musculus*) divided into four groups. Results from each group will be collected on a daily basis. This study will be performed during a period of six months using a total of 100 test subjects (*Mus musculus*) divided into four groups. Results from each group will be collected on a daily basis. Before beginning the study, all mice (AD and healthy) will be pre-screened to measure blood pressure, coordination and balance, etc. Conduct brain scans of normal mice and Stage 1 transgenic Alzheimer mice. Scans measuring brain activity between the two groups will be compared and evaluated. Four groups will be created (each containing 25 subjects)

1). Group A1 will consist of healthy mice that consume standard lab-grade rodent feed.
2). Group A2 will consist of healthy mice that consume a mixture of standard lab-grade rodent feed with 5 mg/kg of freshly ground turmeric added. Two grams of turmeric will be added to every liter of water.

3). Group B1 will contain Alzheimer’s mice and will only consume lab-grade rodent feed.

4). Group B2 will contain Alzheimer’s mice, but will consume lab-grade feed with 5 mg/kg of turmeric added to food and 2 grams of freshly ground turmeric added per liter of drinking water. Each mouse will receive its own separate cage with individual food and water. Amount of food/water consumed will be measured and appropriated for each mouse. All other elements besides food will be kept as constant as possible. SMase activity will be closely monitored (both acidic and basic forms). Plasma levels of SMase and a fluorescent assays will be used to collect enzyme activity. COX-2 activity will be measured as well as reactive oxygen species (ROS). Enzyme activity will be determined using Michaelis-Menten kinetics and evaluated appropriately. Enzymes in blood serum and CSF will also be collected and measured. PLA₂ and ceramide activities will be evaluated for each of the four groups. Data will be statistically evaluated using appropriate parameters.

Mice will be tested periodically every 2 weeks and be subject to a variety of balance/strength/coordination tests. Rotarod test Morris water navigation task, the grip test meter test, maze tests, and a balance beam will all be used. All results will be evaluated using appropriate statistical methods. Extensive collection of data will be conducted throughout the course of the experiment. After a period of six months, all mice will be sacrificed and overall brain appearance will be evaluated by a team of researchers. Brains will be tested for plaques, lesions, demyelination, tissue shrinkage, or any other pathological or abnormal signs.

**Necropsy:** After 60 days, all subjects will be terminated via CO₂ asphyxiation and a necropsy will be preformed to determine the degree to which amyloid-beta plaques have accumulated in the brain.
**Hypothesis:** Mice consuming a turmeric-containing diet will demonstrate lowered susceptibility to development of AD. Also, AD mice on the turmeric diet will show fewer motor/coordination abnormalities than the AD group that did not receive turmeric.

**Prevention of Unnecessary Pain:** If mice display intense pain or suffering, they will be terminated humanely using CO2 asphyxiation.

**Problems/Obstacles:** The most probable foreseen obstacle in this study is whether positive results can be pinpointed to turmeric’s medicinal activity or due to some other unforeseen factor. Although a quantitative evaluation of results will be conducted, this study cannot rule out some other confounding factor that influenced turmeric’s activity. Also, it is unknown whether some component of the lab-grade rat feed exerts a beneficial/negative effect upon turmeric’s activity, both through absorption in the bloodstream and activity in the nervous system. Although environmental factors can be carefully manipulated, there is no guarantee that all conditions for every group will be equivalent.

**Significance of Study:** Many researchers suspect that turmeric possesses incredible potential as a medicinal chemical compound. This must be determined experimentally before turmeric is implemented in a hospital or clinical setting. The future of turmeric as a healthful compound is dependent upon this study and other related investigations.

**Future Directions:** Avenues of future research are largely contingent upon experimental results. If the turmeric-fed mice exhibit retardation of AD progression, future research should determine turmeric’s optimal dosage for Alzheimer’s patients. Although investigating this issue to some extent, this current study will not elucidate all details of effective drug regimen. Also, future studies must determine if turmeric is best taken alone or in combination with another compound. In particular, CP and liquiritin are two compounds are special interest Methods will be suggested as to certain novel compounds that can be screened for medicinal efficiency. Many current studies complain that turmeric is poorly absorbed because of its relatively poor solubility in water.


Discussion

As multiple studies demonstrate, NSAIDs can mediate immune response, but they do not effectively reduce Aβ plaques.\textsuperscript{10} Although well-intentioned, current drug designs suffer from a fundamental flaw in their production-- they are derived largely from empirical methods and do not usually begin with a presuppositional framework in mind. This directly leads to the treatment of specific disease symptoms (COX-2 reduction, thromboxane inhibition, etc.) without targeting the basic cause of the disease. Since these therapies are limited in scope, they also demonstrate numerous secondary effects, some of which are deadly. Although offering great potential, memantine suffers from short-term activity as neural receptors quickly accommodate. Likewise, lithium fails to offer consistently reliable results (and also demonstrates mixed experimental results). Lithium has also garnished concern over its potentially toxic effect to body organs.\textsuperscript{95} Therefore, neither lithium nor memantine can be employed for patient care or for any kind of long-term treatment. In contrast to pharmaceutical compounds, turmeric offers a wide range of healthful effects. Despite some studies that demonstrate poor solubility, research has determined that fortification with LAB significantly increases turmeric’s bioavailability. This finding suggests that turmeric used for AD therapy should be intentionally grown in the presence of LAB and should be consumed in the freshest form possible. Further studies must verify and add further details regarding how LAB increases curcumin’s effectiveness.

The proposed turmeric study will elucidate further details about how turmeric impacts AD murine models. Adjusting for any confounding factors, these findings can be directly applied to human patients. Based on current research, it is hypothesized that turmeric ingestion will greatly increase cognition and memory and also boost coordination and muscle control. Future studies will investigate dosage considerations and whether turmeric is best used in combination with other compounds or taken by itself. Other transgenic model organisms will be tested to determine if turmeric’s effects are confined to one species or can impact many different species. It is presumed that the latter scenario will be proven correct and that each organism will utilize similar cascades and molecular mechanisms in metabolism of turmeric.
Conclusion

Despite the valiant efforts of researchers and physicians, AD still remains an incurable and deadly disease. Impacting millions around the world each year, it is especially prevalent in America and Europe and targets the elderly demographic. AD is a complex, multifactorial disease that is typified by external Aβ plaques and internal NFTs (due to faulty secretase cleavage of APP and excessive tau phosphorylation respectively). Historically, the *Homo sapiens* genome has always displayed a potential for AD development; widespread Alzheimer’s, however, is a relatively recent phenomenon due to longer lifespans and harmful environmental factors. It is hypothesized that upregulation of inflammatory cascades (driven by AA and ceramide production) are the major stimulant in driving disease pathology. A complex interplay of diet, gene expression, and organism variability is implicated in this process. It is believed that this internal inflammation provides the motive force behind SP and NFT accumulation. By means of a vicious cycle, even the body’s own immune response stimulates disease conditions through microglial activation. Likewise, the “cytokine storm” produced by AA and ceramide pathways only increase inflammation, caspase activity, and cell necrosis. Multiple drug regimens have been developed for treating AD, but so far are only mildly effective (or even detrimental in many cases). Although demonstrating some potential, COX-2 and cholinesterase inhibitors are not yet suitable for patient use and their efficacy in treating AD is doubtful. Even NSAIDs (perhaps the most effective of AD therapeutics) and memantine are extremely time-dependent, and their usefulness rapidly declines with each use. In the absence of a useful AD therapeutic, researchers are desperately searching for novel compounds that produce clinical results. As multiple studies show, the burden of evidence weighs heavily on turmeric, warranting the need for further clinical and experimental investigations. It is hoped that turmeric will turn the tide of disease progression and restore cognitive, learning, and STM functions. Additionally, a turmeric-based drug regimen will inhibit neuroinflammation by retroactively preventing exorbitant eicosanoid expression by deactivation of PGH2 synthase (COX-2). Although most neural damage is irreversible, curcumin will demolish existent Aβ plaques, unravel NFTs, and prevent apoptotic
activity.\textsuperscript{118} It is tantamount that turmeric therapies be administered in the earliest stages of the disease since this promises the greatest chance of a full recovery. These conclusions will be investigated further in the “Proposed Study” section, which will test how turmeric affects motor skills, coordination, and balance of transgenic Alzheimer mice. Because of its widespread effects in targeting SPs and NFTs, impacting AA and sphingomyelinase cascades, and halting ROS, turmeric warrants incorporation into Western medicinal practice. It is hoped that turmeric may be used in tandem with another medicinally useful compounds (natural or synthetic) to ebb the tide of AD.\textsuperscript{108} Of all potential therapies, turmeric will halt the Alzheimer epidemic and its ravaging grip on Western society.
References


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**Figures**


