TAU- A FRIEND TURNS FOE

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ABSTRACT

Alzheimer's disease remains an illusive disorder without effective treatment and cure. None of the hypotheses proposed accounts for the gradually progressing deterioration and variable clinical presentations. It encompasses the diversity of neuronal and nonneuronal abnormalities and elucidates how a disorder has the brain as its most vulnerable target organ. For many years Amyloid β was regarded as the main culprit and all the therapeutic efforts were centered towards Aβ, now it seems that centre stage has been shifted towards Neurofrillary tangles and Tau. Tau is a protein associated with microtubules which help in the proper functioning of microtubules. When tau gets hyperphosphorylated it gets detached from microtubules and get aggregated in the form of tangles called neurofibrillary tangles. In addition to the well-known changes in phosphorylation state, tau undergoes multiple truncations and shifts in conformation as it transforms from an unfolded monomer to the structured polymer characteristic of neurofibrillary tangles. Neurofibrillary tangles influence neuronal function in several ways and these changes finally ends with neuronal death and cognitive decline. In this review, we discuss and summarize the physiological roles of tau and how it deviate from its primary role into tangled form and turn a foe in Alzheimer's disease.

Key words: Alzheimer's disease, Memory, Tau, Neurofibrillay tangles, Amyloid β

INTRODUCTION

Alzheimer's disease is a progressive neurological and psychiatric disorder characterized by a progressive decline in memory, cognitive performance and loss of acquired skills leading to apraxia, agnosia and aphasia (1) that ultimately lead to death (2). The cognitive decline in AD is accompanied by neuronal atrophy and loss, mainly in the cortex, hippocampus and amygdale (3). The characteristic pathological features of Alzheimer's disease (AD), still remains relevant that described by Alois Alzheimer in 1906 - namely the senile plaques and neurofibrillary tangles. A major constituent of the extracellular senile plaques is amyloid β, an insoluble peptide derived from abnormal cleavage of amyloid precursor protein (APP). The neurofibrillary tangles represent insoluble fibrous material accumulating within neurons and consist mainly of an abnormally phosphorylated form of tau.

APP is a normal neuronal constituent of unknown function, while tau is a microtubule associated protein. Both amyloid and tau are foci of research on possible causative factors in AD (4, 5). Research on tau is encouraged by reports that the density of tangles correlates better with the dementia of AD than does the appearance of senile plaques (6).The tangles of AD are confined largely to the cortical regions of brain and entorhinal cortex and hippocampus are affected early and severely, followed by the medial temporal, parietal and frontal cortices. The motor and visual cortices show little involvement until very advanced stages (7). The early involvement of the entorhinal cortex, hippocampus and association areas explains the loss of recent memory as the prominent early symptom (8).

ROLE OF TAU IN STABILIZING NEURONAL STRUCTURE

Neurons are functional units of nervous system and these are cells with a complex morphology. Morphological differentiation of a neuron involves the extensive rearrangement of the cytoskeleton, which is responsible for maintaining the cell's shape. The cytoskeleton is composed of three main components: the microtubules, the microfilaments, and the intermediate filaments. Microtubules are very dynamic structures, and in proliferating cells such as neuroblasts (neuron precursors), their probability of assembly is the same as that of depolymerization in all directions.

However, during the differentiation of a neuroblast into a neuron, the microtubules become stabilized in specific directions, thereby generating the cytoplasmic extensions that will become the axon and the dendrites (9). Several proteins serve to stabilize microtubules and such proteins include the microtubule-associated proteins (or MAPs) MAP1A, MAP1B, MAP2 and Tau (10, 11). Being present mainly in the axon of a neuron, tau function and dysfunction have been related to axonal microtubule function, both alone and in synergy with other MAPs (12). In pathological situations, tau has additionally been shown to be capable of forming aberrant fibrillar polymers (13).

Weingarten and colleagues first discovered microtubule-associated protein tau in 1975 as a heat stable protein that facilitates in vitro microtubule assembly (14). Further studies demonstrated that tau is a phosphoprotein and that phosphorylation negatively regulates its ability to stimulate microtubule assembly (15). Subsequently, it was reported that microtubule assembly in brain extracts from AD cases is impaired and that the hyperphosphorylation of tau may contribute to this deficit (16). These findings significantly increased interest in tau and tau phosphorylation both in physiological and in pathological settings.

The density of tau inclusions correlating well with regional and global aspects of cognitive decline and these structures occur in the regions of the brain responsible for

the various cognitive domains that are compromised during the course of AD (17,18).

TAU- A MAJOR COMPONENT OF PHF'S

The breakthrough discovery that catapulted tau into the limelight was the finding that it is the major component of the paired helical filaments (PHFs) that make up the neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) brain, and that the tau in PHFs and NFTs is abnormally phosphorylated (6,7,12).Tau in AD brain, especially in PHFs, is abnormally hyperphosphorylated and glycosylated. At the later stages of tangle formation, the tau is increasingly ubiquinated. In a normal neuron, biological function depends on an intact microtubule network through which much of the axoplasmic transport is supported. The AD abnormally phosphorylated tau (AD P-tau) competes with tubulin in binding to normal tau, MAP1, and MAP2 and inhibits their microtubule assembly-promoting activities. The disruption of the microtubule network probably compromises the axonal transport and starts retrograde degeneration of the affected neurons. The neuronal cytoskeleton in AD is progressively disrupted and is replaced by bundles of PHFs, leading to the formation of neurofibrillary tangles (19).
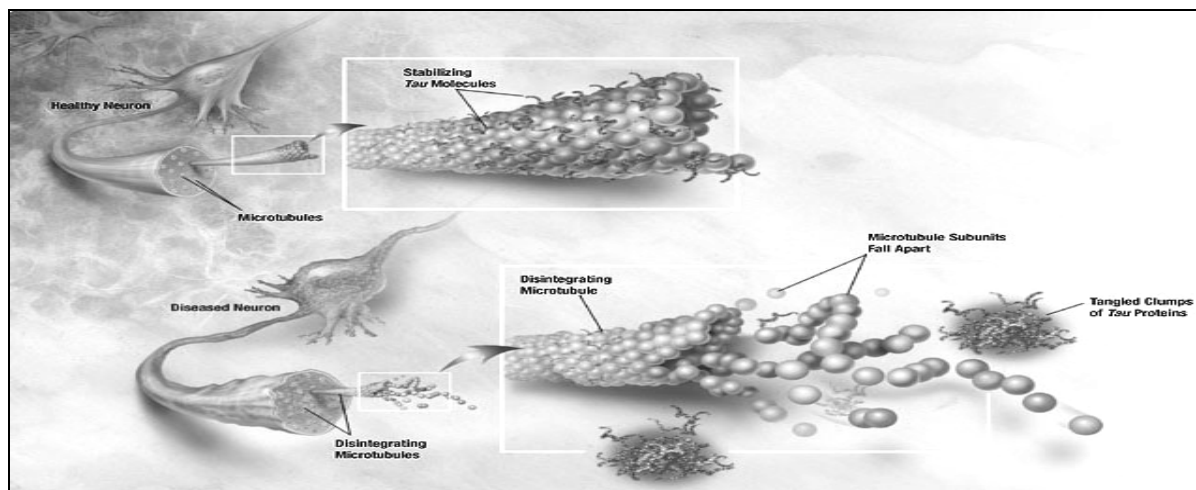
Figure 1:.Disruption of microtubules in AD: Normal healthy neurons contain Tau in bound form that helps in stabilization of Microtubules. Once hyperphosphorylated tau get detached and changes into polymeric, tangled form and leads to degeneration of neurons.

CHANGES AT CELLULAR LEVEL IN TAU PATHOLOGY

The observations of increased mitochondrial components in lysosomes (20) synaptic vesicles failing to arrive at terminals (21, 22) and vesicle accumulation in cell bodies (23) suggest that microtubule- dependent transport of organelles is hindered in AD (24). The clinical importance of these findings is that microtubule reduction may underlie the loss of neuronal connectivity suggested as the basis of cognitive loss in AD (21). Studies supporting the amyloid cascade hypothesis suggest that amyloid is upstream of tau and could be a significant factor in hyperphosphorylating tau, resulting in the formation of neurofibrillary tangles followed by neurodegeneration (25).

TAU HYPERPHOSPHORYLATION AND INFLUENCE OF KINASES

The major function of tau in the CNS is in the stabilization of microtubules in neurons and tau might be involved in the establishment and maintenance of neuronal polarity. The C-terminus of tau binds to axonal microtubules while the N terminus binds to neural plasma membrane components suggesting that tau functions as a linker protein between both. Besides this, tau is also involved in various signal transduction pathways where tau binds with non-receptor src family tyrosine kinases and influences neurite growth and the motility of microtubules in response to extracellular signals (26). Elevated levels of phosphorylated tau correlate with the presence of dynamic microtubules during periods of high plasticity in the developing mammalian brain (27). However, all of these functions of tau are dependent on its ability to be phosphorylated at site-specific epitopes (28). Various kinases and phosphatases are involved in the regulation of tau phosphorylation that occurs at a number of serine, threonine and proline residues (29, 30).

In vitro studies suggest that increases in Aβ production may potentiate tau phosphorylation by activation of kinases such as glycogen synthase kinase-3 (GSK- 3) (31).

The level of tau phosphorylation is the consequence of the action of protein kinases that modify tau protein and that of phosphatases that dephosphorylate the previously modified tau. Several tau kinases have been described and they are grouped into two different types: proline (PDPK) and non-proline (NPDPK) directed protein kinases (32). PDPK modified Ser-Pro or Thr-Pro tau motifs and three main PDPK have been described to phosphorylate tau, GSK3, also known as tau kinase I (33), cdk5 (or tau kinase II) (34,35) and stress kinases like JNK and p38 (36-40).NPDPK modify Ser orThr residueswhere not followed

by prolines. Among them are: cyclic-AMP dependent kinase (41), Ca 2+ /calmodulin dependent kinase (CaMPKII), Casein kinase II, protein kinase C (42) or microtubule affinity regulating kinase (MARK) (43). The stress activated protein kinases SAPK3/p38gamma and SAPK4/p38delta also cause abnormal hyperphosphorylation of tau (27, 44).

Tau hyperphosphorylation is at the crux of most tauopathies since hyperphosphorylation dissociates tau from microtubules, destabilizes them and forms paired helical filaments (PHF) in vitro (15,45). Tau phosphorylation is regulated by an exquisite equilibrium between kinase and phosphatase activities. An imbalance of these two enzymatic processes can result in abnormal hyperphosphorylation of tau and the generation of PHF (30).

In the hippocampus, an inverse relation has been found between the number of eNFT and the number of surviving neurons. It suggests that neurons that degenerate, have previously developed tau aggregates. During normal development, Tau undergoes multiple post translational modifications; phosphorylation, truncation, nitration, glycation, glycosylation and polyamination. The most important modifications are truncation and phosphorylation. Phosphorylated Tau has reduced capability in binding to microtubules and hyperphosphorylation contributes to formation of Tau filaments as observed in Alzheimer's disease (45-47). Normally, tau in adult brain contains 2-3 moles phosphate per moles of Tau but hyperphosphorylated tau from AD contains 5-9 moles of phosphate group per mole of protein (48).

GENETIC INFLUENCE ON TAU HYPERPHOSPHORYLATION

Tau protein is coded by a unique gene (49) that upon alternative splicing of the transcript generates different tau isoforms. In neurons from the central nervous system (CNS) up to six tau isoforms (from 352 to 441 amino acids) are expressed (50,51) by alternative mRNA splicing. Fractionation of CNS tau isoforms by gel electrophoresis indicates the presence of more than the expected six isoforms. This is due to the existence of additional tau isoforms arising by postranslational modification (phosphorylation) of the coded ones (52).

It has been suggested that tau phosphorylation could regulate tau binding to microtubules and in pathological situations to its self aggregation (15). NPDPK phosphorylation mainly occurs at the tubulin-binding region of the tau molecule. Therefore, it has been suggested that this type of modification could result in a decrease in the binding of tau to microtubules as studies of phosphorylation of serine 262 support (53). In pathological situations, like Alzheimer's disease (AD), where phosphorylation of the microtubule binding region takes place, a decreased binding of tau to microtubules has been observed (54).

INSULIN SIGNALING AND TAU

In the brain, insulin serves as a neuromodulatory and neuroendocrine molecule in addition to its usual metabolic function, playing a significant role in neuronal growth and survival (55).

Insulin growth factors are potent neurotrophic agents (56) and protect and rescue hippocampal neurons from amyloid and other toxins (57). IGF-1 can cross the blood brain barrier and is neuroprotective in vivo (58). Further, insulin-sensitizing agents such as troglitazones (thiazolidinediones, TZDs) can have potent neurotrophin-like survival activity (59). All of this contributes to the hypothesis that increased insulin-like signaling in the brain would promote neuronal survival and longevity.

It has been suggested that Aβ could act as an antagonist for the insulin receptor (55). Thus, the PKB kinase should be inactivated and as a consequence of that, GSK3 could be active leading to tau hyperphosphorylation (48).

OXIDATIVE STRESS AND TAU HYPERPHOSPHORYLATION

Oxidative stress has been implicated in AD(60) and consequence of stress is lipid peroxidation. In this way, modification of arachidonic acid yields compounds like 4-hydroxynonenal (HNE) (61) or acrolein (62). Both compounds are present in the brain of AD patients and colocalize with NFT (61,63). Also, HNE inhibits tau dephosphorylation in cultured hippocampal neurons (64) and its adduction to phospho-tau, but not unphosphorylated tau, allows the in vitro formation of filaments and also the appearance of filaments in cultured human neuroblastoma cells (65). Recently, it has been shown that acrolein favors tau phosphorylation by the stress kinase p38 (66). This result suggests that oxidative stress could facilitate tau phosphorylation through the activation of p38 (67).

Furthermore Tau has 0, 1 or 2 N terminal inserts (resulting from the splicing in or out of exons 2 and 3) and either 3 or 4 microtubule-binding domains (resulting from the splicing in or out of exon 10) (68). The splicing of tau is developmentally regulated, as is its phosphorylation state. In fetal brain, only the shortest tau isoform is present (minus exons 2, 3 and 10) and fetal tau is more extensively phosphorylated than adult tau (69). Tau from fetal brain promotes microtubule assembly less efficiently than tau from adult brain (70) and elevated levels of phosphorylated tau correlate with the presence of dynamic microtubules during periods of high plasticity in the developing mammalian brain (27).

The longest form of adult human brain tau has 80 Ser or Thr residues and 5 Tyr residues; therefore, almost 20% of the molecule has the potential to be phosphorylated (71). Site-specific phosphorylation of tau is essential for its proper functioning. Furthermore, there is increasing evidence that inappropriate phosphorylation of tau, which leads to tau dysfunction, results in decreased cell viability. Indeed, in all neurodegenerative diseases in which tau pathology has been observed, the tau is abnormally phosphorylated (72).

CONCLUSION

Both senile plaque and NFT formation are related to aging even among normal patients, however, the frequency of NFT correlates to dementia better than does that of senile plaques. Therefore, it is not surprising that disorders leading to hyperphosphorylated tau accumulation are associated with dementia. Although significant progress has been made in our understanding of how phosphorylation regulates tau function and it is also known that precise, specific and coordinated phosphorylation events are crucial for proper functioning of Tau. Given that the phosphorylation of specific sites on tau can inhibit its ability to bind microtubules efficiently and increase its ability to polymerize, it is likely the abnormal phosphorylation events play a role in tau pathogenic processes. It is also likely that the phosphorylation-induced loss of function (i.e. impairment of microtubule binding), as well as the toxic gain of function (i.e. an increased propensity to oligomerize), synergize to reduce the levels of functional tau and thus disrupt normal microtubule-based functions, which could contribute to the demise of the cell. Both amyloid and tau hypotheses have their merits and demerits but further studies should be carried on to clarify the role of abnormal tau phosphorylation in the pathogenic cascades in AD, as well as the protein kinases that directly phosphorylate tau in both the normal and disease states.

REFERENCES

1. Stuchbury G, Munch G, Alzheimer's associated inflammation potential drug targets and future therapies, J Neural Transm,112,2005, 429–453,

2. Yankner BA, New clues to Alzheimer's disease: unraveling the roles of amyloid and tau, Nature,2,1996,850–852,

3. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, HymanBT, Neuronal loss correlates with but exceeds neurobrillary tangles in Alzheimer's disease, Ann Neurol,41,1997, 17-24,

4. Selkoe DJ, Cell biology of the beta-amyloid protein and the genetics of Alzheimer's disease, Cold Spring Harbor Symposiaon Quantitative Biology, 61,1996,587-596.

5. Iqbal K, Grundke-Iqbal I, Molecular mechanism of Alzheimer's neurofibrillary degeneration and therapeutic intervention, Annals of the New York Academy of Sciences,77,1996, 132-138.

6. Braak H, Braak E, Evolution of the neuropathology of Alzheimer's disease, Acta Neurologica Scandinavica Supplementum,165,1996,3-12.

7. Brun A, Englund E, Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading, Histopathology,5(5),1981,549-564.

8. Edith G, McGeer, Patrick L, Innate Inflammatory Reaction of the Brain in Alzheimer Disease, MJM,3,1997,134-141.

9. Mitchison T, Kirschner M, Cytoskeletal dynamics and nerve growth, Neuron,1,1988, 761–772

10. Matus A, Microtubule-associated proteins: their potential role in determining neuronal morphology, Annu Rev Neuroscience, 11, 1988,29–44.

11. Binder LI, Frankfurter A, Rebhun LI,The distribution of tau in the mammalian central nervous system, J Cell Biol,101,1985,1371–1378.

12. Gonzalez-Billault C, Engelke M, Jimenez-Mateos EM, Wandosell F, Caceres A, Avila J, Participation of structural microtubule-associated proteins (MAPs) in the development of neuronal polarity, J Neurosci Res,67,2002,713–719.

13. Cleveland DW, Hwo SY, Kirschner MW,Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly, J Mol Biol,116,1977, 227–247.

14. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW, A protein factor essential for microtubule assembly, Proc Natl Acad Sci,72,1975,1858–1862.

15. Lindwall G, Cole RD, Phosphorylation affects theability of tau–protein to promote microtubule assembly, J Biol Chem,259,1984,5301–5305.

16. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI, Abnormal phosphorylation of the microtubule-associated protein (tau) in Alzheimer cytoskeletal pathology, Proc Natl Acad Sci,83,1986,4913–4917.

17. Ghoshal N, Garcia-Sierra F, Wuu J, Leurgans S, Bennett DA, BerryRW, BinderLI, Tau conformational changes correspond to impairments of episodic memory in mild cognitive impairment and Alzheimer's disease, Exp Neurol,177,2002,475–493.

18. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT, Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease, Neurology, 42,1992,631–639.

19. Kidd M, Alzheimer's disease: an electron microscopical study, Brain,87,1964,307–320.

20. Hirai K, Aliev G, Nunomura A, Figioka H, Russdl RL, Atwood CS, Johnson AB, Kress Y, Vinters HV ,Tabaton M, et al, Mitochondrial abnormalities in Alzheimer's disease, J Neurosci,21,2001,3017–3023.

21. Scheff SW, DeKosky ST, Price DA, Quantitative assessment of cortical synaptic density in Alzheimer's disease, Neurobiol Aging,1990,1129–37.

22. Terry RD, The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis, J Neuropathol Exp Neurol,55,1966,1023–1025.

23. Praprotnik D, Smith MA, Richey PL, Vinters HV, Perry G, Filament heterogeneity within the dystrophic neurites of senile plaques suggests blockage of fast axonal transport in Alzheimer's disease, Acta Neuropathol, 91,1966,226–235.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
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24. Suzuki K, Terry RD,Fine structural localization of acid phosphatase in senile plaques in Alzheimer's presenile dementia, Acta Neuropathologica, 8,1967,276–284.

25. Hardy J, Selkoe DJ,Medicine – The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science, 297,2002,353–356.

26. Buee L, Bussiere T, Buee-Scherrer V, Delacourte AH, Tau protein isoforms phosphorylation and role in neurodegenerative disorders, Brain Res Brain Res Rev,2000, 3395– 130.

27. Brion J P, Octave J N, Couck A M, Distribution of the phosphorylated microtubule-associated protein tau in developing cortical neurons, Neuroscience,63,1944, 895-909.

28. Quadros A, Ophelia I, Ghania A,Role of tau in Alzheimer's dementia and other neurodegenerative Diseases, J Appl Biomed, 2007, 51–12.

29. Butler M, Shelanski ML, Microheterogeneity of microtubule-associated tau proteins is due to differences in phosphorylation, J Neurochem, 47,1986,1517–1522.

30. Ferrer I, Gomez-Isla T, Puig B, Freixes M, Ribe E, Dalfo E, Avila J, Current advances on different kinases involved in tau phosphorylation and implications in Alzheimer's disease and tauopathies, Curr Alzheimer Res,2005,23–18.

31. Alvarez G, Munoz-Montano JR, Satru SJ, Avila J, Bogonez E, Dıaz-Nido J, Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration, FEBS Letters 1999; 453: 260–264,

32. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Titani K, Ihara Y, Proline-directed and non-proline-directed phosphorylation of PHF-tau, J Biol Chem, 270,1995, 823-829.

33. Munoz-Montano JR, Moreno FJ, Avila J, Diaz-Nido J, Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons, FEBS Lett,411,1997,183-188.

34. Liu F, Iqbal K, Grundke-Iqbal I, Gong C X,Involvement of aberrant glycosylation in phosphorylation of tau by cdk5 and GSK-3 beta, Febs Letters,530,2002,209-214.

35. Yamaguchi H, Ishiguro K, Uchida T, Takashima A, Lemere CA, Imahori K, Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I glycogen synthase kinase-3 beta and cyclin-dependent kinase 5 a component of TPK II, Acta Neuropathol Berl, 92,1996, 232-241.

36. Goedert , Hasegawa M, Jakes R, Lawler S, Cuenda A, Cohen P, Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases, FEBS Letters, 409,1997, 57-62.

41. Johnson GV, Differential phosphorylation of tau by cyclic AMP-dependent protein kinase and Ca2+/calmodulin- -dependent protein kinase II: metabolic and functional consequences, Journal of Neurochemistry,59,1992,2056-2062.

42. Correas I, Díaz-Nido J, Avila J, Microtubule-associated protein tau is phosphorylated by protein kinase C on its tubulin binding domain, J Biol Chem,267,1992, 15721-15728.

43. Trinczek B, Biernat J, Baumann K, Mandelkow EM, Mandelkow E, Domains of tau protein differential phosphorylation and dynamic instability of microtubules, Mol Biol Cell,6,1995, 1887-1902.

44. Buee-Scherrer V, Goedert M, Phosphorylation of microtubule-associated protein tau by stressactivated protein kinases in intact cells, FEBS Lett,515,2002,151–154.

45. Alonso A, Zaidi T, Grundke-Iqbal I, Iqbal K,1994, Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer– disease, Proc, Natl Acad Sci, 91,1994, 5562–5566.

46. Alonso A, Zaidi T, Novak M, Grundke I, Iqbal K, Hyperphophorylation induces self assembly of tauinto tangles of paired helical filaments /straight filaments, Proc Natl Acad Sci,98,2001,6923-6928.

47. Gorath M, Stahnke T, Mronga T, Golbaum O, Ichter – Landsberg, Developmentl changes of Tau protein and mRNA in cultured rat brain oligodendrocytes,Glia,36,2001, 89-101.

48. Kopke E, Tung YC, Shaikh S, Alonso A, Iqbal K, Grundke I,Microtubule associated protein tau:abnormal phosphorylation of non paired filament pool in Alzheimer's disease, J Bio Chem,268,1991,24374-24384.

49. Reynolds CH, Utton MA, Gibb GM, Yates A, Anderton BH, Stress-activated protein kinase/c-Jun N-terminal kinase phosphorylates tau protein, Journal of Neurochemistry, 68,1997, 1736-1744.

50. Reynolds CH, Betts JC, Blackstock WP, Nebreda AR, Anderton BH, Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: differences in vitro between the mitogen-activated protein kinases ERK2 c-Jun N-terminal kinase and P38 and glycogen synthase kinase-3beta, J Neurochem,74,2000,1587-1595.

51. Zhu X, Rottkamp CA, Boux H, Takeda A, Perry G, SmithMA, Activation of p38 kinase links tau phosphorylation oxidative stress and cell cycle-related events in Alzheimer disease, J Neuropathol Exp Neurol, 59,2000, 880-888.

52. Zhu X, Raina AK, Rottkamp CA, Aliev G, Perry G, Boux H, Smith MA, Activation and redistribution of c-jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease, J Neurochem,76,2001, 435-41.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
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53. Biernat J, Gustke N, Drewes G, Mandelkow EM, Mandelkow E, Phosphorylation of ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubulebinding, Neuron, 11,1993,153–163.

54. Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X, Raina AK, Vinters HV, Tabaton M, Johnson AB et al, Microtubule Reduction in Alzheimer's disease and Aging is Independent of tau Filament Formation, Am J Pathol,162,2003,1623-1627.

55. Gasparini L, Xu H, Potential roles of insulin and IGF-1 in Alzheimer's disease, Trends Neurosci, 26,2003, 404–406.

56. Carson MJ, Behringer RR, Brinster R,L, McMorris FA, Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice, Neuron,10,1993, 729–740.

57. Dore S, Kar S, Quiron R, Insulin-like growth factor I protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity, Proc, Natl, Acad, Sci, 94,1997,4772–4777.

58. Liu X, Yao DL, Webster H,Insulin-like growth factor I treatment reduces clinical deficits and lesion severity in acute demyelinating experimental autoimmune encephalomyelitis, Multiple Sclerosis,1,1995, 2–9.

59. Nishijima C, Kimoto K, Arakawa Y, Survival activity of troglitazone in rat motoneurones, J, Neurochem,76,2001,383–390.

60. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, SmithMA,Oxidative damage is the earliest event in Alzheimer disease, J Neuropathol Exp Neurol,60,2001,759-767.

61. Sayre LM, Zelasko DA, Harris PLR, Perry G, Salomon RG, Smith MA,4-hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease, J Neurochem, 68,1997, 2092-2097.

62. Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E, Acrolein is a product of lipid peroxidation reaction Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins, J Biol Chem,273,1988,16058-16066.

63. Lovell MA, Xie C, Markesbery WR, Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures, Neurobiol Aging, 22,2001,187-194.

64. Mattson MP, Fu WM, Waeg G, Uchida K,4 hydroxynonenal a product of lipid peroxidation inhibits dephosphorylation of the microtubule-associated protein tau, Neuroreport,8,1997, 2275-2281.

65. Perez M, Hernandez F, Gomez-Ramos A, Smith M, Perry G, Avila J, Formation of aberrant phosphotau fibrillar polymers in neural cultured cells, Eur J Biochem,269,2002,1484-1489.

66. Gomez-Ramos A, Diaz-Nido J, Smith MA, Perry G, Avila J, Effect of the lipid peroxidation product acrolein on tau phosphorylation in neural cells, J Neurosci Res,71,2003,863-870.

67. Odetti P, Garibaldi S, Norese R, Angelini G, Marinelli L, Valentini S, Menini S, Traverso N, Zaccheo D, Siedlak S, Perry G, Smith MA, Tabaton M, Lipoperoxidation is selectively involved in progressive supranuclear palsy, J Neuropathol Exp Neurol, 59,2000,393-397.

68. Johnson G V, Jenkins S M, Tau protein in normal and Alzheimer's disease brain, J Alzheimers Dis, 1,1999,307-328.

69. Watanabe A, Hasegawa M, Suzuki M, Takio K, Morishima- Kawashima M, Titani K, Arai T, Kosik K S, Ihara Y, In vivo phosphorylation sites in fetal and adult rat tau, J Biol Chem,268,1993,25712-25717.

70. Yoshida H, Ihara Y, Tau in paired helical filaments is functionally distinct from fetal tau: assembly incompetence of paired helical filament-tau, J Neurochem, 61,1993,1183-1186.

71. Goedert M M, Spillantini R, Jakes D, Rutherford R, CrowherRA, Multiple isoforms of human micrortubule-associated protein-tau: sequences and localization in neurofibrillery tangles of Alzheimer's-disease, Neuron,1989, 3519-526.

72.LeeVM, GoedertM, Trojanowski JQ,Neurodegenerative tauopathies, Annu Rev Neurosci, 24,2001,1121-1159.

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