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Formaldehyde as a Trigger for Protein Aggregation and Potential Target for Mitigation of Age-Related, Progressive Cognitive Impairment

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Abstract: Recently, formaldehyde (FA), existing in a number of different cells including neural cells, was found to affect age-related cognitive impairment. Oral administration of methanol (the metabolic precursor of FA) triggers formation of senile plaques (SPs) and Tau hyperphosphorylation in the brains of monkeys with memory decline. Intraperitoneal injection of FA leads to hyperphosphorylation of Tau in wild-type mouse brains and N2a cells through activation of glycogen synthase kinase-3β (GSK-3β). Furthermore, formaldehyde at low concentrations can directly induce Tau aggregation and amyloid β (Aβ) peptide deposits *in vitro*. Formaldehyde-induced Tau aggregation is implicated in cytotoxicity and neural cell apoptosis. Clarifying how FA triggers Aβ deposits and Tau hyperphosphorlyation will not only improve our understanding of the molecular and cellular mechanisms of age-related cognitive impairment but will also contribute to the ongoing investigation of alternate targets for new drugs. Here, we review the role of FA, particularly that of endogenous origin, in protein aggregation and as a potential drug intervention in the development of age-related cognitive impairment.

Keywords: Aggregation, Alzheimer's disease, amyloid β, aspartame, formaldehyde (FA), hyperphosphorylation, methanol, Tau protein.

1. INTRODUCTION

Formaldehyde (FA, MW 30), one of the first organically active compounds to appear during the Earth's early evolution [1], is found throughout the universe [2], and in low concentrations on earth in the clouds, air, soil, and oceans. Formaldehyde may have originated from photochemical reactions in the Earth's primitive atmosphere, which mainly consisted of molecular nitrogen, water vapor, carbon dioxide, and trace amounts of molecular hydrogen and carbon monoxide [3].

Besides its natural existence, FA presents in a wide variety of man-made products and anthropogenic emissions. To a certain extent, our living environment is surrounded by extrinsic FA, and it is also ubiquitous in living organisms, such as microorganisms, plants, and animals [4], formed as an intermediate in the metabolism of endogenous amino acids and in the one-carbon cycle [5]. Despite this exogenous and endogenous FA exposure, FA does not accumulate in the organism because it is highly reactive and can bond to many other organic compounds or be metabolized quickly and converted to formic acid and CO_2 in the human body.

The most common form of dementia, Alzheimer's disease (AD) [6, 7], is now the paramount focus of research in the field of neurodegenerative diseases [8, 9]. Although the pathogenesis of AD is poorly understood, its typical pathological changes have been elucidated: the extracellular amyloid deposits of aggregated Aβ [10-12] and intracellular neurofibrillary tangles (NFTs) [13, 14]. Aggregated Tau proteins are a major component of the latter [15, 16]. Hyperphosphorylation of Tau appears to proceed to protein aggregation, paired helical filaments (PHFs), and the consequent NFTs [17], which has been documented as a product induced by abnormal phosphorylation in the earlier stage of age-related cognitive impairment [18-22].

However, what triggers hyperphosphorylation of Tau, deposits of Aβ, and onset of dementia needs to be investigated. Recent studies of environmental factors that may be responsible for sporadic AD exhibiting the above pathological traits have paid great attention to both exogenous and endogenous FA because of its non-genetic pathogenesis. Formaldehyde is involved in triggering abnormal phosphorylation of Tau protein, promoting Aβ deposits and cognitive impairment. Therefore, formaldehyde is proposed as a bio-

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marker to diagnose and a potential drug target to therapeutically intervene with age-related cognitive impairment [23].

2. SOURCES OF HUMAN FORMALDEHYDE

2.1. Exogenous Formaldehyde

Formaldehyde in the human body comes from different sources (Fig. **1**), which can be divided into exogenous and endogenous pathways [24]. For exogenous FA, the primary uptake is from a limited number of foods. Foods such as fruits, vegetables, and alcoholic beverages are likely the main sources of exogenous methanol in the healthy human body [25]. However, the safety of food additives should be taken into consideration. According to The US Center for Disease Control and Prevention, AD's age-adjusted death toll soared to 100 times its rate in 1981 (Supplementary Fig. **1**). Aspartame, a sweetener containing methanol, was first added to food in 1981. Methanol is a rare component of the human diet, but changes in food choices and the introduction of aspartame have increased the average consumption gradually over the last 40 years, mirroring the increase in incidence in AD over the same time period [26] (Fig. **2**).

Methanol, with only a handful of significant dietary sources, is reliably found in just one of the major food groups, fruits and vegetables, where it is chemically locked safely to pectin, which can pass digestion without absorption by the gut. When fruit, vegetable, or other juices are heat processed and packaged for distribution, however, methanol is released over time from pectin's strong methyl ester bond, and the free methanol is trapped in the container, readily available for quick absorption upon consumption. Ready-to-serve juice drinks have become very popular with an increasingly health-conscious public since traditional carbonated beverage companies introduced them in the late 1970s as a "healthy" alternative in vending machines. These juice drinks would, by now, have been considered the major source of methanol in the American diet if not for the extraordinarily rapid and massive introduction of aspartame, which reliably releases 10% of its weight as methanol within minutes of consumption [27].

Whether the methanol from aspartame can trigger Tau phosphorylation needs further investigation. The recent dietary introduction of aspartame, 11% methanol by weight, has greatly increased methanol consumption in humans [25]. Aspartame is metabolized by gut esterases and peptidases to three common chemicals: aspartic acid, phenylalanine, and methanol [27]. Metabolic methanol may also occur as a result of fermentation by gut bacteria to produce FA [28]. Shindyapina and colleagues displayed an increase in concentrations of MeOH and FA in volunteers' blood plasma when consuming citrus pectin, ethanol, and red wine [29].

The other source of FA is from respiration when exposed to air polluted with high concentrations of the FA present in a wide variety of products such as some plywood adhesives, abrasive materials, insulation, insecticides, and embalming fluids (Environmental Protection Agency USA, "Toxicological Review of FA Inhalation Assessment" June/18/2010). The major sources of anthropogenic emissions of FA are motor vehicle exhaust, power plants, and manufacturing plants that produce or use FA or substances that contain it such as petroleum refineries, coking operations, incinerating, wood burning, and tobacco smoke.

2.2. Endogenous Formaldehyde

Formaldehyde existing in various cells [5] may have some unknown physiological functions and may be related to some substantial human diseases. The homeostasis of FA metabolism depends on the balance of its production and degradation. The physiological concentration of brain FA is 0.2-0.4 mM as reported using either gas chromatography/mass spectrometry [30] or fluorescence high-performance liquid chromatography (HPLC) [31]. However, it is about 0.1 mM determined by absorbance of 2,4-dinitrophenylhydrazine (2,4-DNPH) on HPLC [32].

Endogenous FA is produced in multiple pathways. Methanol metabolism is one of the major ways to produce FA in liver cells, neural cells, and other cells [27]. Demethylation of DNA, RNA, and histone produces FA catalyzed by DNA methyltransferases (DNMTs) [33, 34], fat mass and obesity-associated protein (FTO) [35, 36], and lysine(K)

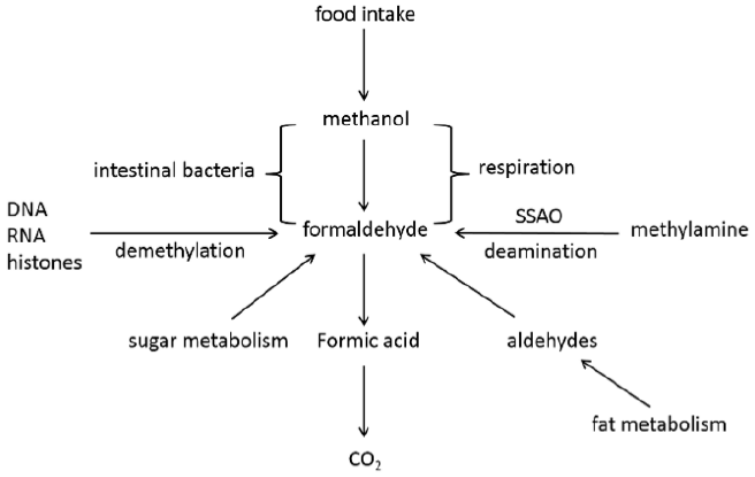

Fig. (1). Resources of endogenous formaldehyde. Endogenous formaldehyde comes from multiple pathways [36, 68].

demethylase (KDM) [37], respectively. Production of FA was demonstrated in the pathway from N5-methyltetrahydrofolate to tetrahydrofolate [38, 39]. Formaldehyde also resulted from oxidative stress. For instance, a remarkable increase in the concentration of uric FA was observed after surgery [40]. Adipocytes, vascular endothelial cells, and smooth muscle cells are rich in semicarbazide-sensitive amine oxidase (SSAO) that generates FA. Methylamine is deaminated to FA catalyzed by SSAO under oxidative stress [41].

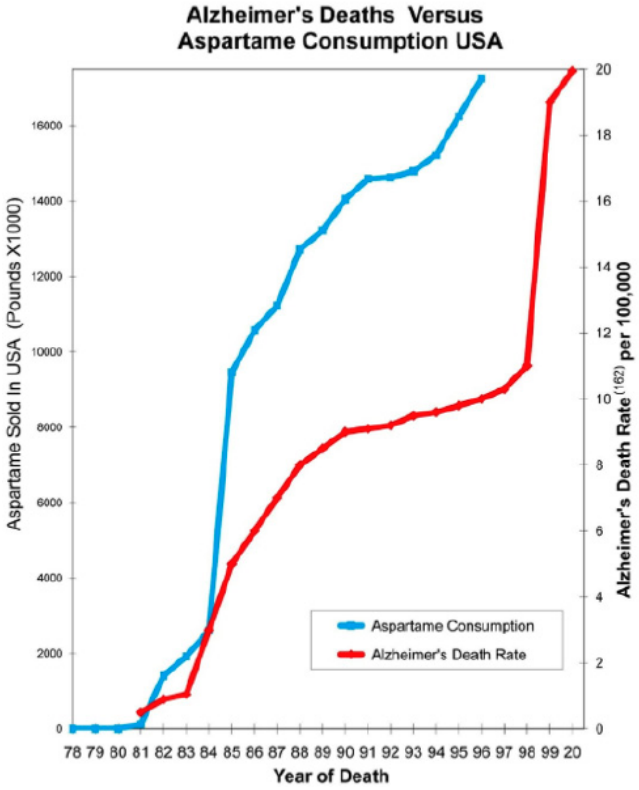

Fig. (2). Increase in US age adjusted Alzheimer's death rate as aspartame (methanol) consumption increases.

Furthermore, formaldehyde, malonaldehyde, acetone, acetaldehyde, and propionaldehyde [42], the degradation products of oxygen-derived free radicals, all act as presumptive markers for lipid peroxidation (LPO) [43, 44], and those products of LPO and other lipid peroxidation accumulate in aging rat and human brains [45-47]. Previous work has shown that certain substrates, such as nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine, will promote FA production by rat nasal cytochrome P-450-dependent monooxygenases [48].

Formaldehyde is degraded mainly via enzyme pathways. There are three groups of isozymes for FA degradation: alcohol dehydrogenase I (ADH1), alcohol dehydrogenase III (ADH3 or ADH5 [49], and aldehyde dehydrogenase 2 (ALDH2) [50]. Formaldehyde is transformed either to CO2, which is expired by the lungs, or to formic acid, which is excreted via urine. Even though formaldehyde is quickly metabolized in liver and other cells, formaldehyde is also rapidly produced from different pathways *in vivo* and this maintains its metabolic homeostasis [32].

3. FORMALDEHYDE AND HUMAN COGNITION

3.1. Formaldehyde and Cognitive Ability

Whether FA plays a physiological role in the normal metabolism remains an interesting and controversial question. An epidemiological study in the Japanese population indicated a high risk for late-onset Alzheimer's disease with deficiency in mitochondrial aldehyde dehydrogenase, the enzyme responsible for catalyzing FA to formic acid [51]. Lee and his colleagues investigated the role of FA increase in S-adenosyl-L-methionine (SAM) induced Parkinson's disease-like disorders [52]. Recently, Wang *et al.* showed that post-operative cognitive damage resulted from surgery stress, combined with an elevated FA concentration [40].

High FA levels could also be a risk factor for cognitive impairment in older adults. Excess brain FA resulting from either exogenous or endogenous pathways can induce animal memory impairment [53-56] and cognitive decline in humans [57-59], which is highly correlated with the recent discovery that the pathological FA concentration, detected by fluorescent techniques, approaches 0.4-0.5 mM in AD-transgenic mice and clinical AD patients [60].

3.2. Exogenous Formaldehyde and Cognitive Impairment

Abundant evidence indicates that excess FA impairs memory. Exposure of rats to exogenous gaseous FA induces the accumulation of FA [61], decreases the number of hippocampal neurons [62], and leads to memory decline [55]. Similar results were also shown in mice administrated FA either through intraperitoneal injection or gavage [31, 63]. The long-term oral administration of FA to monkeys induced a significant decline of working memory [64]. Epidemiological investigations indicate that exogenous FA exposure causes human cognitive decline and is associated with neurofilament protein changes and demyelization in hippocampal neurons [57-59, 65, 66].

3.3. Endogenous Formaldehyde and Age-Related Cognitive Impairment

Chen *et al.* observed that ADH3-KO drosophila, with its considerably decreased FA, exhibited a decline in the ability to learn and in memory, suggesting lack of endogenous FA can affect animal cognition [67]. As hypothesized recently, endogenous FA is involved in learning and memory, in particular learning ability because FA acts as a donor for the methylation of DNA, RNA, and histones [37, 68]. Also, high concentration of formaldehyde inhibited the N-methyl-D-aspartate receptor, which functions in learning and memory [69, 70]. Homeostasis of endogenous FA metabolism may benefit from these methylations, which are involved in cognitive behaviors in learning and memory [71, 72].

It appears that endogenous FA, whose concentrations increase with aging (>70 years) [71], is involved in the progression of age-related cognitive impairment. The brain FA of 8-month-old wild-type (C57) mice is higher than at 3 months. Two-year old SD rats have a significantly higher concentration of FA in their hippocampi than 1-month-old animals. Qiang and her coworkers found the imbalance of FA metabolism of senescence accelerated mouse-prone 8 strain

(SAMP8), cognitively impaired from the age of 3 months [73]. Furthermore, a high level of hippocampal FA was observed in the autopsies of Alzheimer's patients after death.

In clinical trials, uric FA positively correlated with the severity of cognitive impairment of Alzheimer's patients [31]. Yu and colleagues estimated the correlation between uric FA and general cognitive abilities in a community-based elderly population, and measured the extent and direction in which the correlation varied with demographic characteristics [74]. Their results demonstrated a negative impact of endogenous FA on general cognitive abilities. Their data also showed a correlation between concentrations of endogenous formaldehyde and education levels: the higher the education levels, the lower the concentration of uric formaldehyde for normal community-based elderly subjects.

4. FORMALDEHYDE AND PROTEIN AGGREGATION

Amyloid deposits in senile plaque and aggregated Tau protein are two typical pathological features of AD [75]. Clarification of what triggers brain protein aggregation is a scientific imperative for understanding age-related cognitive impairment. As described previously, we believe that the ability to metabolize FA declines with aging. FA accumulation occurs and becomes an important cause of senile chronic cognitive failure [76].

Hua *et al.* found that FA at low concentrations can induce Tau aggregation, hindering Tau's role in the assembly and stabilization of the microtubule system [77, 78]. In in-vitro experiments, FA induced-Tau oligomers showed globular-like aggregates with cytotoxicity resulting in neuronal dysfunction and even cell death [79, 80]. Observing the effects of aldehydes derived from oxidative deamination and oxidative stress on beta-amyloid aggregation, Yu and coworkers (2004) proposed that increased semicarbazide sensitive amine oxidase (SSAO)-mediated deamination many contribute to protein deposition, formation of plaques, and inflammation, and thus may be involved in the pathophysiology of chronic vascular and neurological disorders, such as diabetic complications, atherosclerosis, and Alzheimer's disease [81]

4.1. Formaldehyde and Tau Hyperphosphorylation

Protein phosphorylation is the addition of a phosphate group by esterification to a protein at three amino acids: serine, threonine, and tyrosine. Phosphorylation is the most common posttranslational modification for Tau [82]. Increases in Tau phosphorylation reduce its ability to form microtubules, which results in neuronal cytoskeleton destabilization [83, 84]. There are 85 putative phosphorylation sites on Tau protein. Many kinases and phosphatases are involved in the regulation of Tau phosphorlyation, and each phosphorylation site may be subjected to the action of one or more protein kinases [85-87]. Tau kinases are grouped into three types: protein kinases PDPKs (proline-directed protein kinases), protein kinases non-PDPKs, and protein kinases specific for tyrosines [88]. An increase in the number of phosphorylated sites in a Tau molecule and/or in the number of phosphorylated Tau molecules at a given site can be termed Tau hyperphosphorylation. Tau hyperphosphorylation can also result from inhibition of related phosphatases [89, 90]. The activities of various protein kinases and phosphatases mainly determine the balance of the Tau phosphorylation level [91], whose disruption contributes to the abnormal Tau phosphorylation observed in the brains of AD patients.

Oral administration of low concentrations of methanol leads to hyperphosphorylation of Tau in the brains of mice [63]. In experiments with cell lines, Tau was hyperphosphorylated, not only in the cytoplasm but also in the nucleus of neuroblastoma (N2a) cells in the presence of FA [92] (Fig. **3**).

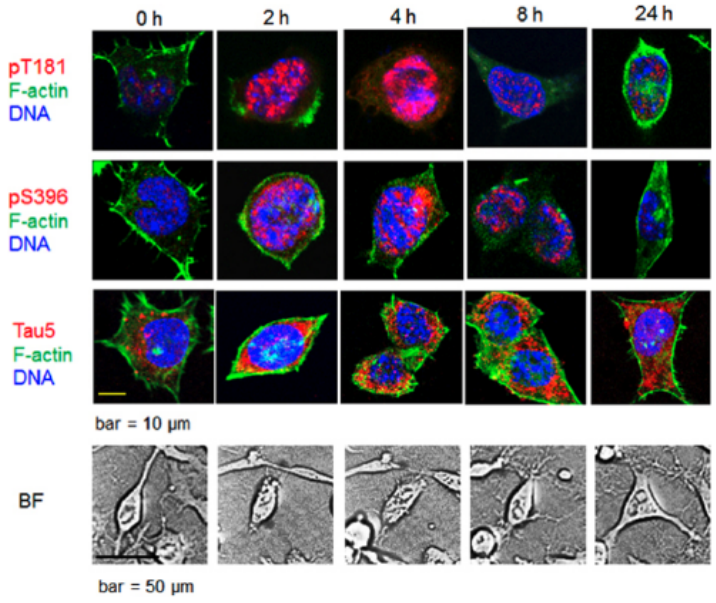

Fig. (3). Tau phosphorylation in N2a cells after treated with FA. Phosphorylated Tau was detected by anti-pT181 and anti-pS396 antibodies, and total Tau was assayed with Tau5 antibody in the presence of FA. Nuclei and F-actin were stained with Hoechst-33258 (blue) and phalloidin (green), respectively. The signals of anti-pT181 and anti-pS396 strikingly increased in nuclei of N2a cells in 4 hours after FA treatment, and most of them were rarely co-localized with the DNA staining. A small part of Tau5 signals were co-localized with DNA except for most of them [92].

Western blot has revealed phosphorylated Tau polymers in the cell nucleus. Intraperitoneal injection with FA has also demonstrated that Tau is phosphorylated at T181, S396, and T231 in both the cortex and hippocampus. Polymers of phosphorylated Tau were also detected in the nuclei of the mouse brain. The concentration of brain formaldehyde significantly increased after mice were injected with formaldehyde through the tail vein as shown in Fig. (**4**) [93]. In further experiments with mice, both oral administration with methanol and intraperitoneal injection with FA showed a significant decline of cognitive abilities.

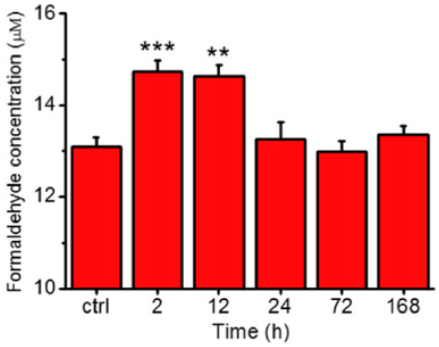

Fig. (4). Changes in concentrations of formaldehyde in mouse brain through tail vein injection of formaldehyde. The concentration of brain formaldehyde significantly increased from 2 h to 12 h after mice were injected with formaldehyde through the tail vein under these experimental conditions (n = 5; means ± S.E.M; **p < 0.01, ***p < 0.0001) [93].

Significant accumulation of glycogen synthase kinase-3β (GSK-3β) in the nucleus of N2a mouse brain cells, and elevation of its phosphorylation at Y216, was observed under formaldehyde stress. FA-induced Tau hyperphosphorylation was blocked in the presence of LiCl and CT99021, inhibitors of GSK-3β, and by RNAi interference. Therefore, formaldehyde may cause age-related memory loss by triggering Tau hyperphosphorylation via GSK-3β catalysis and inducing polymerization of Tau. Significantly, then, further investigation of FA-induced Tau hyperphosphorylation may provide novel insights into mechanisms underlying tauopathies.

4.2. DNA Loss of Tau Protection in the Presence of Formaldehyde

Tau protein has been associated with DNA double-strands *in vitro* and *in vivo* [94, 95]. Tau suppresses DNA duplication, but not RNA transcription [96]. It has been proposed that Tau, in addition to promoting and stabilizing microtubule systems, acts as a buffer protein that protects DNA against the attack of free radicals when cells are under oxidative stresses [97]. The complex of Tau associated with DNA features in a sphere-like structure disintegrates when Tau is hyperphosphorylate in the presence of formaldehyde [98] (Fig. **5**). Hyperphosphorylation of Tau at Thr181 and Ser396 was observed in FA-treated N2a cells, and the co-localization of the nuclear phosphorylated Tau protein and DNA was greatly affected by FA treatment, compared to the control without treatment [93]. Additionally, Tau protein phosphorylated by GSK3β showed a retarded interaction with DNA in an electrophoretic mobility shift assay. These findings reveal that hyperphosphorylation may reduce Tau protein's DNA protection, which can lead to fragile DNA, increased neural cell death, and consequent cognitive impairment [98].

Tau is hyperphosphorylated in the presence of FA [98] (Fig. **5**). Hyperphosphorylation of Tau at Thr181 and Ser396 was observed in FA-treated N2a cells, and the co-localization of the nuclear phosphorylated Tau protein and DNA was greatly affected by FA treatment, compared to the control without treatment [93]. Additionally, Tau protein phosphorylated by GSK3β showed a retarded interaction with DNA in an electrophoretic mobility shift assay. These findings reveal

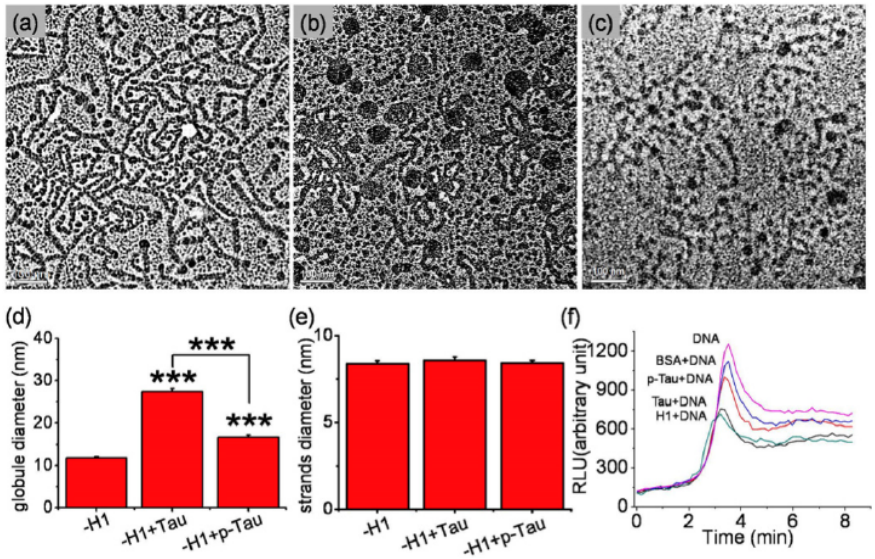

Fig. (5). Visualization of Tau associated with DNA by electronic microscopy. Images represent DNA without histone H1 as control (panel **a**), DNA with unphosphorylated tau in globule-like complexes (panel **b**), DNA with p-Tau (panel **c**), a column plot to indicate the diameters of folded DNA chains (penal **d**), and the diameters of unfolded DNA chains (panel **e**). Ability of tau to prevent peroxidation of DNA as described [98]. (panel **f**). Bars = 100 nm, ***p < 0.001, n = 30.

that hyperphosphorylation may reduce Tau protein's DNA protection, which can lead to fragile DNA, increased neural cell death, and consequent cognitive impairment [98].

4.3. Formaldehyde Inducing Amyloid-Beta Deposits in Monkey Brains

A key aspect of Alzheimer's is beta-amyloid plaque, sticky clumps of an abnormal protein in the brain. Except for Tau hyperphosphorylation, however, administration of FA either by oral intake or intraperitoneal injection did not induce senile plaque in the brain of wild-type mice [63, 93]. The structure of mouse APP may be different from the human's [99]. So far, formation of senile plaque (amyloid-beta deposits) has not been clearly observed in the brains of aging wild-type mice except in the transgenic mouse with human APP genes [100]. Yang and her colleagues [64] found Aβ deposits in both the hippocampus and cortex of the monkey brain (3-5 years old, *Macacamulatta)* after daily oral feeding of a low concentration of methanol dissolved in water for 1 to 2 years (Fig. **6**). Methanol is converted to FA in liver and brain vascular cells [101]. Furthermore, administration of low concentrations of methanol, as a precursor of FA, induces not only memory loss and Tau hyperphosphorylation but also formation of senile plaque in monkey brains [64]. This suggests that FA may be involved in the formation of Aβ deposits in human brains.

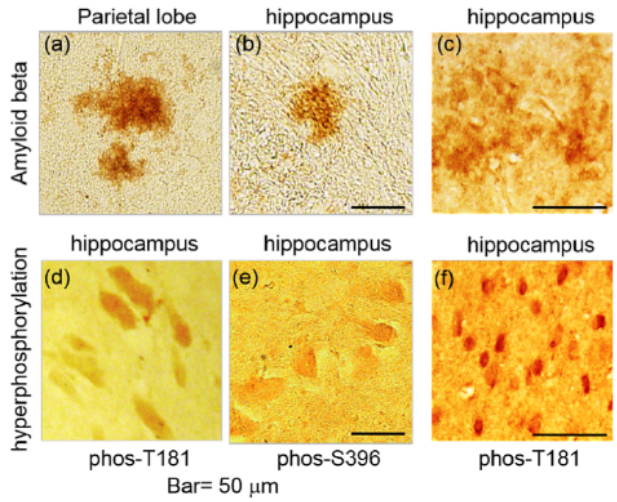

Fig. (6). Formaldehyde triggers abnormal modification of amyloid β and Tau protein. Five year-old monkeys (*Macaca mulatta*) who were administrated with low concentrations of methanol for 2 years and suffered from senile plaque (panel **a,b**), Aβ deposits (panel **c**), neurofibrillary like-tangles in parietal lobe and hippocampus (panel **d,e,f**) [64].

Formaldehyde stress acts as one of the important factors in age-related cognitive impairment, which is related with Tau hyperphosphorylation and Aβ aggregation [102].

5. FORMALDEHYDE AS A BIOMARKER AND POTENTIAL DRUG TARGET

5.1. Formaldehyde Scavenger in Protection of Cells

It has been suggested that reducing agents may decrease the concentration of FA. Recently, Miao and her colleagues compared the effects of epigallocatechingallate (EGCG), Resveratrol (RES), glutathione (GSH), and dimedone (DMD) on the level of FA in the culture of N2a cells (Fig. **7a**) [103]. Among these, RES was most effective in decreasing the concentration of FA in the cultural medium, showing protection of the N2a cells from FA impairment (Fig. **7b**). But the strong reductant GSH had no effect on the decrease of FA or protection of the cells. Evidently, not all reductants can suppress the concentration of FA.

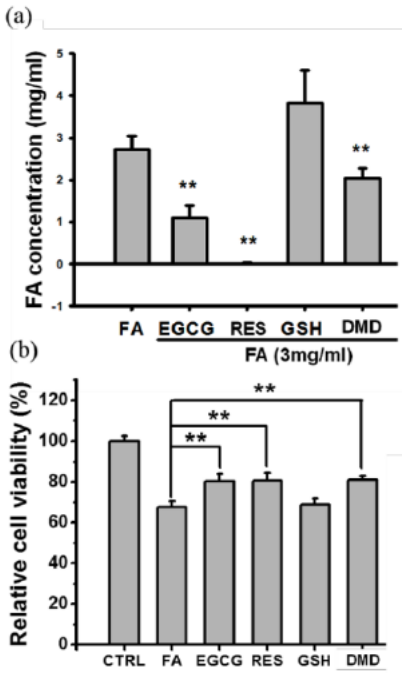

Fig. (7). Formaldehyde scavenger can rescue SH-SY5Y cells in the presence of formaldehyde. (**a**) Formaldehyde solution was incubated with EGCG(2), RES(3), GSH(4) and DMD(5) (final concentration was 0.01 mM for each reagent) for 40 min, respectively, and then formaldehyde concentration was detected by HPLC. Formaldehyde (3 g/L) without any scavengers (1) was used as control (n = 3; MEAN±SD; **P< 0.01). (**b**) Cell viability examined with CCK-8 assay. Cells were under the treatment with 0.2 mM FA and EGCG, RES, GSH and DMD (final concentration 0.01 mM for each agent) for 24 h, respectively. 1: Cells without treatment of formaldehyde as a blank control; 2: Cells only treated with 0.2 mM formaldehyde as a negative control; 3: Cells treated with formaldehyde and EGCG; 4: Cells treated with formaldehyde and RES; 5: Cells treated with formaldehyde and GSH; 6: Cells treated with formaldehyde and DMD [103]. (n = 3; MEAN±SD; **P< 0.01).

To further establish whether resveratrol can rescue the memory loss of SD rats induced by chronic excess FA treatment, Tong and his coworkers injected normal adult SD rats intraperitoneally with 0.5 mM FA (60 mg/kg, i.p.) for 30 consecutive days. The excess FA suppressed hippocampal LTP *in vivo*. The amplitude of fEPSP in these groups (140%) was markedly lower than that (160%) of SD rats injected (i.c.v.) with excess FA. Significantly, learning and memory retrieval ability declined in the FA-treated group. Resveratrol, as an exogenous FA scavenger [104], substantially rescued (P<0.01) rat hippocampal LTP suppression caused by excess FA. It markedly reduced (P<0.01) excess FA-induced cognitive decline and decreased levels of hippocampal FA.

5.2. Suppression of Tau Phosphorylation with Icariin

Excess FA activates Tau kinase GSK-3β by enhancement of Tyr216 phosphorylation in the mouse brain, leading to Tau hyperphosphorylation and aggregation [93]. To investigate the effects of neuroprotective drugs on FA-treated cells, Li and her colleagues in the 1st Hospital of Peking University used icariin (ICA) [105, 106], a flavonoid found in the Chinese herbal medicine Epimedium that exhibits a neuroprotective activity [107]. They observed cell viability, apoptosis, and morphological changes by using CCK-8 assay, flow cytometry, and confocal microscopy, respectively [108, 109]. They found that icariin (1–10 μM) prevents FA-induced cell death in SH-SY5Y cells in a dose-dependent manner, with the optimal effect observed at 5 μM. As shown with western blot, icariin suppresses FA-induced phosphorylation at Thr181 and Ser396 of Tau protein, while having no effect on the expression of the total Tau protein level. Icariin reduced Tyr216 phosphorylation and increased Ser9 phosphorylation. Their results demonstrate that icariin is able to prevent FA-induced injury in SH-SY5Y cells, possibly through the inhibition of GSK-3β-mediated Tau phosphorylation.

5.3. Geniposide in Protection of Formaldehyde-Exposed Neural Cells and Suppression of Aβ Deposits

The herbal medicine Tong Luo Jiu Nao (TLJN) contains geniposide (GP) and ginsenoside Rg1 at a molar ratio of 10:1, which has been shown to strengthen brain function in humans and improve learning and memory in animals through ameliorating blood supplies and vessels. Because chronic FA damage of the central nervous system has been presented as a risk factor for age-associated cognitive dysfunction, Hua and her colleagues have investigated the protective effect of both TLJN and GP in neuron-like cells exposed to FA [110]. They found that TLJN can reverse neuronal damage caused by FA and that its main ingredient, GP, has a major role in this efficacy.

Hua *et al.* have investigated whether TLJN is clinically efficacious in the treatment of dementia and improving learning and memory, specifically whether TLJN reduces amyloidogenic processing in Alzheimer's disease animal models. When aggregated into oligomeric Aβ species, Aβ is known to be able to induce cellular toxicity as well as cognitive impairment. In their experiment, TLJN improved cell viability, inhibited LDH release from neurons, and promoted the outgrowth of neurites of hippocampal neurons treated with Aβ [111].

Geniposide could increase the cell viability of SY5Y-APP695sw cells. The cytotoxicity of pretreated Aβ with geniposide was decreased in a dose-dependent manner. Geniposide is able to protect neurons from Aβ-induced damage, preventing AD-type neuropathology by decreasing Aβ oligomers through accelerating the Aβ fibrillogenesis. Together, this suggests GP and TLJN may be a potential treatment for patients with dementia.

5.4. The Benefits of Regularly Water Consumption

Weight loss is a major clinical feature of Alzheimer's disease [112]. Related chronic dehydration is regarded as a common symptom for patients with age-related cognitive impairment, in particular those with Alzheimer's disease [113]. Formaldehyde and methanol are easily dissolved in water and their removal from the blood depends upon renal function. Chronic dehydration in older patients may result from their diminished perception of thirst and loss of memory, while dehydration causes accumulation of cytotoxic metabolic compounds such as endogenous FA worsening cognitive impairment. This interaction between dehydration and cognitive impairment creates a vicious circle [114].

Chronic dehydration induces renal injury [115]. Regularly drinking water not only relieves chronic dehydration for aging people but also significantly decreases the concentration of endogenous FA including other metabolic compounds, which may offer protection of the central nervous system. Therefore, the habit of regularly drinking water should be encouraged in aging people to help mitigate age-related cognitive impairment at an early stage.

5.5. Ethanol at Low Dose as a Competitive Inhibitor of Methanol's Conversion

Methanol is itself relatively harmless, but its first metabolite, FA, may pose more of a danger. Humans metabolize methanol differently than all other animals except some non-human primates [116]. For a non-human test animal to respond as humans do to methanol poisoning, the dosage of methanol in their diets must be increased high enough to saturate their peroxisomes [27]. Otherwise, animals deal with methanol's conversion to FA within the relative safety of the hundreds of peroxisomes contained in each of their cells, where FA's conversion to formic acid and then carbon dioxide can proceed via the same catalase enzyme. Unlike animals, humans lack a protective mechanism within their peroxisomes due to a yet undefined genetic defect of our catalase detoxification complex that prevents human catalase from metabolizing methanol [116].

Methanol's conversion to FA in humans is left, then, to the free-floating Alcohol Dehydrogenase 1 (ADH 1) expressed in the cytosol of only a handful of non-hepatic cells, which include cells lining the circulatory system of the human brain [117]. Further oxidation of the FA produced in the cytosol is unlikely as it is dependent on finding available aldehyde dehydrogenase. This exposes all organelles, the nucleus of these cells, and the cells in close proximity to these circulatory cells to highly reactive FA that can readily react with proteins and cross-link nucleotides. Since AD is considered a perivascular disease, this association is clearly important.

Ethanol in low concentrations acts as a powerful competitive inhibitor [116] with a 16:1 preference for ethanol to acetaldehyde over the conversion of methanol to FA by ADH I [117]. For this reason, ethanol is the preferred antidote for accidental methanol poisoning in emergency rooms throughout the world. Very low levels of ethanol in the bloodstream substantively prevent all FA production from dietary methanol by the ADH I sites found in the intima, media, and adventitia lining of the circulatory system of the heart and brain, FA that would otherwise be expected to diffuse into the localized tissue.

This protection from FA production may account for the yet unexplained dose region of apparent improvement in the U-shaped curve of alcohol consumption. Epidemiologic studies show moderate consumption of alcohol is associated with a reduced risk of both dementia [117] and myocardial infarction [118]. Recent studies of individuals who consumed at least one alcoholic drink per day show subjects had an additional 86 percent reduction in risk of myocardial infarction if genetically endowed with a genotype of ADH I that was 2.5 times slower to metabolize ethanol than the control. These findings were "consistent with the hypothesis that a slower rate of clearance of alcohol enhances the beneficial effect of moderate alcohol consumption on the risk of cardiovascular disease" [119].

A compelling explanation of the dose region of adverse effects of the U-shaped curve with high ethanol consumption, which shows increased risk of these same diseases, could be the mechanism by which humans habituate to high consumption of ethanol. The induction of the P450 hepatic microsomal ethanol-oxidizing system [120] results in a considerably higher clearance rate of ethanol from the bloodstream for an extended period of time, thus accounting for more consumption leading to statistically less time of protection [25]. Small amounts of supplemental alcohol not sufficient to induce P450 might be expected to prolong the residence time and avoid gaps in the protection afforded by ethanol in preventing methanol-produced FA.

It appears that the average person, whether or not an alcohol drinker, may typically have endogenous ethanol in the blood [121] produced by gut fermentation [122]. This ethanol must pass through the liver via the hepatic portal vein coincidently with dietary methanol absorbed from the gut contents. The liver has the highest concentration of ADH I in the body. Even traces of ethanol in the blood, however, would seem to indicate the absence of available sites remaining for the oxidation of the much less competitive methanol, allowing most dietary methanol to pass freely into the general circulation and then the brain.

In the vasculature of the brain [123] and other ADH I positive organs, the consequences may be similarly troublesome. The closest ADH I free to service the methanol will convert it to FA. If this happens in the liver, where there are ample supplies of aldehyde dehydrogenase, metabolism to carbon dioxide will proceed safely. In the brain, however, methanol-placed FA could become a problem. Formaldehyde is a very reactive chemical with methanol, providing its only easy avenue into the brain.

In fact the only important ADH I to be found in the human brain is located within the vessels [123]. The highest concentration of ADH I is found within the media cells of the vessel walls which is located past the epithelial lining of these vessels and therefore already past the blood brain barrier of the arterial blood flow [113]. These sites would present formaldehyde directly to brain cells surrounding these vessels which is important since AD is primarily a perivascular disease. The potential for disease involvement of these ADHI sites have been, until now, ignored by the scientific community.

5.6. Uric Formaldehyde as a Biomarker

Molecules containing amino groups, such as serum protein, are prone to react with blood FA, disturb the determination, and generate an unacceptable analytic error. Because of the highly reactive chemical behavior of blood FA, the smallest of aldehydes, serum samples must be analyzed quickly after blood is taken from the subject. Urine, on the other hand, is not rich in protein and other reactive compounds that interfere with its analysis, making uric FA a better biomarker.

Various studies using urine as a biomarker have shown that aged patients suffering from post-operative cognition dementia had a high level of uric (endogenous) FA [40]; that levels of uric FA are positively correlated with age-related cognitive impairment [31]; that concentrations of endogenous FA are negatively correlated with the education levels of normal elderly subjects in Beijing communities both urban and rural [72]. This research demonstrates that uric FA can be properly employed as a non-invasive biomarker for age-related cognitive impairment. However, as an indirect biomarker for the nervous system, uric FA should be combined with symptomology, brainnetome [123], and other methods to examine the level of age-related cognitive impairment [124, 125].

As aging, the onset of human metabolic disorders becomes more related and vulnerable to endogenous and exogenous environmental pathogenic factors than genetics (Fig. **8**). As shown in Table **1**, different environmental factors have been found to disrupt the binding affinity of Tau to microtubules or DNA and diminish the stability of its cellular skeleton by up-regulating Tau phosphorylation and amyloid β. However, the direct relation between these environmental factors and neurodegenerative diseases is still waiting to be clarified [126]. Developing techniques and methods specific to Tau hyperphosphorylation and amyloid β issue may help with the early diagnosis of and effective intervention with AD [124, 127].

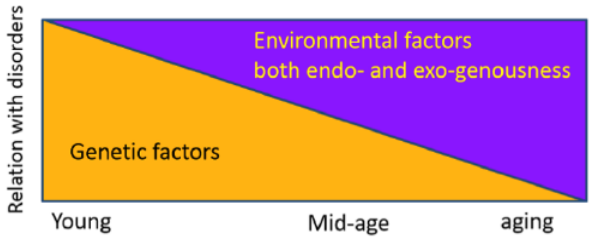

Fig. (8). A putative relation between risk factors and metabolic disorders. Since the onset of age-related cognitive impairment occurs during aging (≥ 65 years old), Alzheimer's disease should be more related with environmental risk factors, for instance the exogenous and endogenous formaldehyde.

CONCLUSION

Age-related cognitive impairment can be divided into two stages: the progressive preclinical stage (pre-mild cognitive impairment and mild cognitive impairment) and the clinical stage (Alzheimer's disease). Producing drugs of long-term and significant efficacy for patients with Alzheimer's disease at the clinical stage has proved difficult

Table 1. Exogenous and endogenous environmental factors inducing Tau hyperphosphorylation and amyloid β.

Tau Hyperphosphorylation		Amyloid β	
Exogenous	**Endogenous**	**Exogenous**	**Endogenous**
Injury and operations [152]	Aggregated protein [153]	Methanol [60, 61]	Apolipoprotein E4 [154]
Drug usage: anesthesia [155]	Formaldehyde [89]	Air pollution [156]	Amyloid precursor protein [157, 158]
Formaldehyde [74]	Metal ions [159]	Formaldehyde [75, 76]	Formaldehyde [160]
	Insulin imbalance [161, 162]		Cholesterol [163, 164]
	Inflammation [165, 166]		Immune response [167, 168]
Food ingredient [169]	Tumor [170, 171]	Food ingredient [172]	Hypertension [173]

Some of exogenous and endogenous environmental factors including FA can trigger Tau hyperphosphorylation. Hyperphosphorylation induces Tau misfolding and aggregation, disassociation from microtubules and DNA [93, 98, 174], which result in neurodegenerative diseases such as AD. Amyloid beta (Aβ), a peptide whose accumulation in amyloid plaques is a hallmark of Alzheimer's disease (AD), disrupts synaptic vesicles [140, 175], normal cellular trafficking, with clathrin-mediated endocytosis being specifically vulnerable. Protein aggregation is influenced by these significant risk factors and some others that involve aging [121,122] and vascular brain injury [176, 177].

[128]. Thus, interventions (including those that regulate endogenous FA metabolism) for aged people with mild or pre-mild cognitive impairment at the preclinical stage should be emphasized. Drug intervention or other therapy could aid recovery for more people at this stage [129, 130]. Although what triggers the onset and progression of AD is now unknown and may be extremely complicated, cognitive impairment resulting from both exogenous and endogenous environmental factors, such as oxidative stress [131-133], heavy metals [134, 135], protein aggregations [136-138], RNA dysfunction [139], synaptic disorders [140], drugs [24,141,142], Apo E [143, 144], down regulation of energetic metabolism [145-147] and ribose metabolic imbalance [148-150] except for formaldehyde [151], should be seriously considered and investigated.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material is available on the publishers web site along with the published article.

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