Review Article
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Possible Prevention of Alzheimer's Disease by Aldehyde Dehydrogenase: A Perspective Review

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Abstract

Investigating the mechanism of neuronal death in Alzheimer's disease is difficult, because only a tiny percentage of neurons are degenerating at any time point during the long prodromal period. Epidemiological, genetic, biochemical and animal model studies have attributed excessive aldehyde load as a cause of Alzheimer neuronal death. Focusing on toxic aldehydes will help fill gaps in our knowledge that cannot be explained by the amyloid β or tau hypotheses. Hydroxynonenal is formed by peroxidation of membrane lipids and LDL or during deep-frying of vegetable oils. It carbonylates Hsp70.1, a heat shock protein with the dual functions of a chaperone protein and lysosomal stabilizer. Hydroxynonenal-mediated Hsp70.1 carbonylation followed by calpain-mediated cleavage of carbonylated Hsp70.1, causes lysosomal neuronal death (the 'calpain-cathepsin hypothesis'). Aldehyde dehydrogenase (ALDH) participates in the removal of not only ethanol-derived acetaldehyde, but also linoleic acid-derived hydroxynonenal. This review describes how scavenging hydroxynonenal by ALDH enzymes prevent Alzheimer's disease.

Keywords: Aldehyde dehydrogenase; Acetic acid bacteria; Calpain-cathepsin hypothesis; Carbonylation; Hsp70.1; Lysosome; Neuronal death

Abbreviations

ALDAs: Aldehyde Dehydrogenase Activators; ALDA-1: N-(1,3-Benzodioxol-5-ylmethyl)-2,6-dichlorobenzamide; ALDH2: Aldehyde dehydrogenase 2; AKR: Aldo-Keto Reductase; GST: Glutathione-S-Transferase; Hsp70.1: Heat-Shock Protein 70.1; HNE: Hydroxynonenal; LDL: Low Density Lipoprotein; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; PUFAs: Polyunsaturated Fatty Acids

Introduction

Currently, Alzheimer's disease is the most common form of dementia affecting more than 50 million people globally [1]. Alzheimer's disease is a chronic neurodegenerative disorder that is characterized by memory impairment, cognitive dysfunction and behavioral disturbances. This disease is nowadays approaching epidemic proportions with neither cure nor preventative therapy available [2]. It is moreover predicted that Alzheimer's disease will affect 115.4 million people in the world by the year 2050 [3]. Alzheimer's disease is pathologically characterized by severe neuronal loss especially in the hippocampus and associated neocortical regions, but the mechanism underlying neuronal death has been unknown for more than a century [4]. For nearly three decades, amyloid β peptides were believed to be the principal cause of neuronal death, but the biomolecular role of amyloid β has not been elucidated in detail. Further, extensive research in the past decade, showing an absence of correlations between amyloid β deposits and widespread neuronal loss in the human autopsy brain and also between amyloid and behavioral abnormalities in animal models, failed to supply convincing evidence that amyloid β is the real culprit of Alzheimer's disease. Most importantly, PET (Positron Emission Tomography) scans showed that considerable amyloid β deposits are not infrequently observed in the cognitively normal individuals, whereas some patients with Alzheimer's disease show no amyloid β deposition [5,6]. Because of the less significant correlations between the extent of amyloid β deposition and either degree of neuronal loss or dementia, we are now obliged to conclude that the amyloid β hypothesis has become less reliable [7-10].

As numerous papers unfruitfully supported a role for amyloid β as a direct cause of Alzheimer's disease, no therapies in clinical use satisfactorily target this molecule [11]. The only currently approved treatments are acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine, as well as the N-methyl-D-aspartate receptor antagonist, memantine, which are thought to improve cognition by targeting specific symptoms of the disease. However, these drugs failed to modify the underlying pathology, i.e., neuronal death itself [12,13]. Given the growing Alzheimer epidemic, there is an urgent need to discover alternative, more effective therapies that can slow down or halt Alzheimer neuronal death. Since the brain is rich in Polyunsaturated Fatty Acids (PUFAs) and harbors a relatively high concentration of oxygen in the lipid bilayer, both oxidative stress-induced lipid peroxidation and accumulation of aldehyde products, especially hydroxynonenal, are key factors in neurodegeneration. By reducing membrane integrity, hydroxynonenal inhibits the proteasome function, triggers protein accumulation, inhibits electron transport chain activity, reduces the Krebs cycle activity and increases lysosomal and mitochondrial membrane permeability to eventually cause programmed cell death [10] (Figure 1).

Previous epidemiologic studies have indicated that long-term use of certain Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) show a protective effect on the development of Alzheimer's disease [14-16]. However, this evidence was based only on observational studies and the exact mechanism remains unclear because different NSAIDs have different effects on Alzheimer's disease [17]. Agents that diminish the formation of hydroxynonenal and accelerate its removal, may
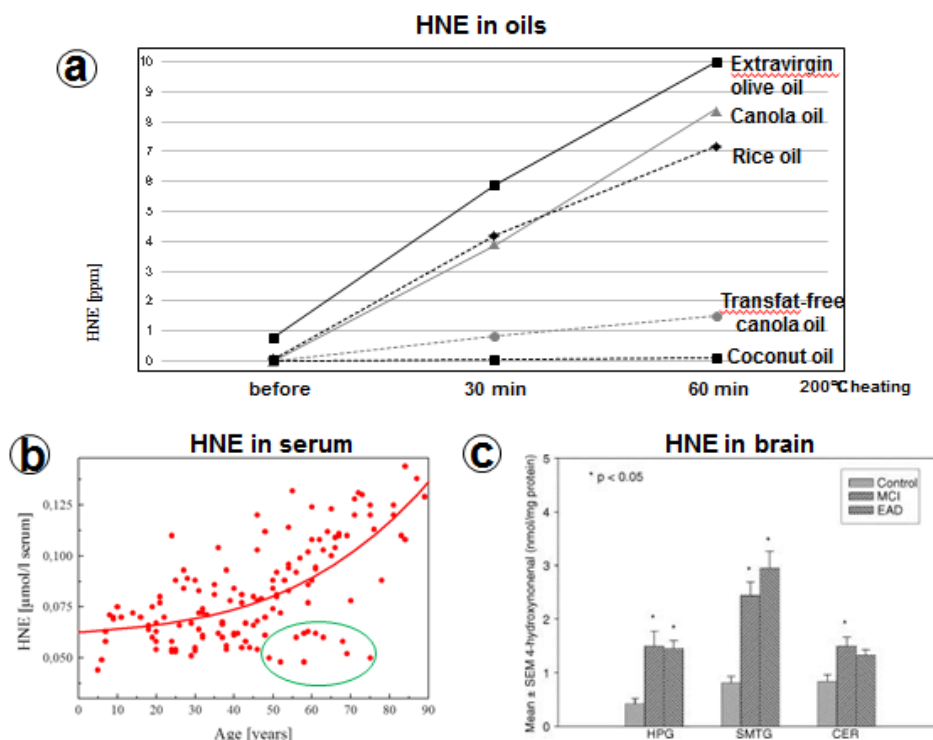
Figure 1: Generation (a), serum level (b) and brain concentration (c) of hydroxynonenal (HNE)

a: HNE generation (ppm) in five representative five cooking oils at 30 and 60 min during 200°C heating. Extra virgin olive oil generates abundant HNE during heating, because it contains ~10% of linoleic acid. Intriguingly, the product from Australia contained a small amount of HNE prior to heating, because of warm temperature within the ship's container en route to Japan. Popular can ola oil and rice oil containing abundant linoleic acid generate a large amount of HNE, and both soybean and sunflower oils are similar (data not shown here). In contrast, transfat-free canola oil generates very little, and coconut oil comprising of saturated fatty acids generates negligible HNE.

b: Age-dependent increase of the serum HNE concentration in the healthy subjects [64]. Note that some elderly subjects (green circles) shows a significantly low serum levels, presumably due to ALDH $2^{+/+}$, ApoE2 inheritance, or very little ω-6 PUFA consumption.

c: HNE concentration in the Hippocampal Gyrus (HPG), Superior and Middle Temporal Gyrus (SMTG) and the Cerebellum (CER) is significantly (p<0.05) higher in patients with mild cognitive impairment (MCI) and Early Alzheimer's Disease (EAD), compared to control subjects [90].

provide a possible therapeutic mode of intervention to prevent or reduce Alzheimer neuronal death. A mitochondrial enzyme, aldehyde dehydrogenase 2 (ALDH2), is the rate-limiting enzyme in the metabolism of ethanol, oxidizing acetaldehyde to acetic acid in the liver [18,19]. ALDH2 also participates in metabolizing hydroxynonenal and was reported to promote cytoprotective mechanisms in ischemia/reperfusion injury by the active removal of hydroxynonenal [20-24]. Accordingly, agents such as the ALDH2 activator, Alda-1 and one of the NSAIDs, flurbiprofen, that increases the enzyme activity of ALDH2 and thereby help scavenge hydroxynonenal, must have a potential for slowing down or preventing the disease progression [25,26]. Here, by considering hydroxynonenal as the real culprit of Alzheimer's disease, the author perspectively reviews the most reliable preventative strategy.

Explaining Alzheimer Neuronal Death by the 'Calpain-Cathepsin Hypothesis'

Since Ca^{2+} plays a significant role in neuronal function, dysregulation of Ca^{2+} may lead to the degenerative neuronal death as shown by the calcium hypothesis of Alzheimer's and brain aging [27]. Investigating the mechansim of neuronal death in Alzheimer's disease is extremely difficult, because only a tiny percentage of neurons is degenerating at any given time point in a very long disease process of up to 20 years. Since neurons maintain large volumes of membrane

and cytoplasm, they must continually traffic damaged/aged/misfolded proteins long distance from the distal ends of axons and dendrites back to the perinuclear cell body where lysosomes await their recycling [28]. Accumulation of these garbage proteins in the post-mitotic cells without recycling is particularly detrimental for the cell survival, because the control of protein turnover is crucial for the maintenance of neurons [29]. Thus, the proper functioning of lysosomes is indispensable for the survival of neurons.

Whilst previously considered simply as the final destination for cellular waste products intended for breakdown, it is becoming increasingly apparent that the lysosome is closely involved in programmed cell death [30]. The lysosome may initiate or enhance cell death programmes as a result of permeabilization/rupture of its limiting membranes and the subsequent leakage of cathepsins into the cytosol, where they can act as 'executioner proteases'. Therefore, aberrant lysosomal function, as revealed by granulo-vacuolar degenerations or autophagic vacuoles on microscopy, should be a key factor influencing the gradual progression of neurodegeneration in Alzheimer's disease [31-33]. The first evidence of lysosomal membrane destabilization was reported over 40 years ago for cell death occurring in vitro, but interests in the role of lysosome in necrotic cell death gradually faded during the following decades [34]. However, in the past two decades, lysosomal cell death has been widely accepted to occur *in vivo* under both

physiological and pathological conditions. An exquisite physiological example of non-apoptotic, lysosome-mediated programmed cell death is the post-lactational regression (involution) of the mammary gland to remove alveolar mammary epithelium and return the gland to its pre-pregnant state. This is one of the complex, highly-regulated cell death programmes which occur in the adult mammalian organism [35-37]. Milk fat globules during mammary gland involution upon cessation of lactation are known to be toxic to epithelial cells, since perturbation of lysosomal vesicle membranes by high levels of free fatty acids being derived from milk triglycerides, results in the controlled leakage of cathepsins, which culminates in the physiological cell death [37].

The molecular cascade that controls release of lysosomal cathepsins to initiate cell death in disease, was first elucidated using the ischemic monkey experimental paradigm by the author and colleagues [38,39]. In 1998 Yamashima et al. ultrastructurally confirmed evidence of the lysosomal membrane rupture in the hippocampal CA1 neurons after transient ischemia and formulated the 'calpain-cathepsin hypothesis' as a mechanism of ischemic neuronal death [39]. Recently, heat-shock protein 70.1 (Hsp70.1, also called Hsp70 or Hsp72) was found to have the dual functions of not only a molecular chaperone, but also as a stabilizer of the lysosomal limiting membrane [40-43]. Since neurons cannot dilute noxious components through cell division, autophagy followed by lysosomal degradation is indispensable for cell survival [44]. Hsp70.1 not only modulates protein folding and trafficking of garbage proteins to lysosomes, but also helps lysosome-dependent degradation of damaged/aged/misfolded proteins [45,46]. In the postischemic CA1 neurons of the same monkey experimental paradigm, Oikawa et al. found by proteomics that the carbonylative

oxidation occurs at the key site Arg469 of Hsp70.1 [47]. This was essentially consistent with similar data from rats fed a high fat diet containing ethanol, where Hsp72 (compatible with human Hsp70.1) modified by hydroxynonenal showed adduct formation at Cys267 in its ATPase domain [48]. It is suggested by these data that Hsp70 may become dysfunctional by the hydroxynonenal-mediated modification (Figures 2a and 2b).

Both oxidative stress and cerebral ischemia have been thought to be the representative causative factors of Alzheimer's disease [49-54]. Long-standing oxidative stress and decreased cerebral blood flow due to aging, combined together, contribute to late-onset/age-related occurrence of neuronal death in the elderly. Fischer et al. reported a significant reduction of vascular net density in the basal forebrain and the hippocampus of patients with Alzheimer's disease [55]. Not only acute extensive ischemia during stroke but also chronic mild ischemia due to vascular reduction and/or arteriosclerosis due to aging, may induce activation of the Ca^{2+} dependent, papain-like protease, calpain (EC 3.4.22.17). For instance, Taniguchi et al. found that μ-calpain is activated more than 7-fold in the brains of patients with Alzheimer's disease, compared to the age-matched controls [56]. This is presumably because the former showed more pronounced ischemia due to vascular reduction compared to the latter, and chronic ischemia and amyloid β deposition synergically facilitate calpain activation [32,33,57]. Intriguingly, Hsp70.1, especially after carbonylation by hydroxynonenal, is prone to cleavage *in vitro* by activated calpain and the cleaved Hsp70.1 is no longer able to traffick damaged/aged/misfolded proteins for recycling [58,59] (Figures 2a and 2c).
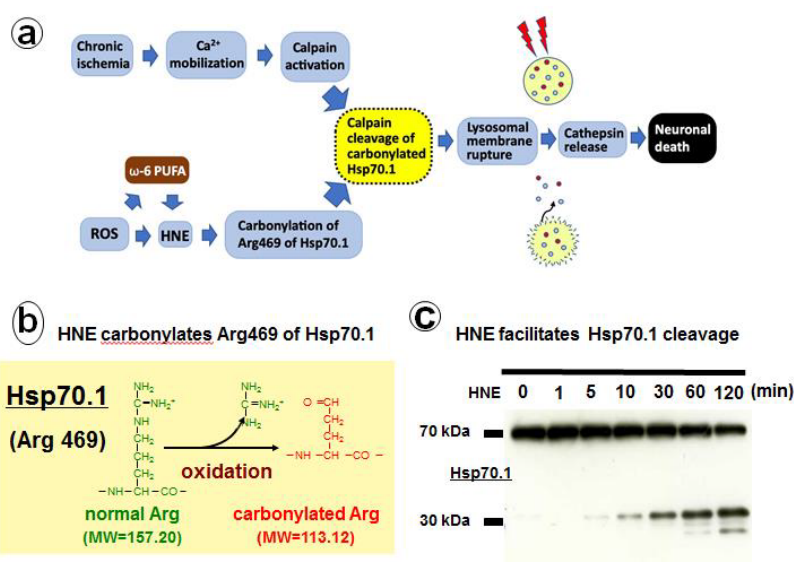

Figure 2: The 'calpain-cathepsin hypothesis' (a), carbonylation (b) and cleavage of Hsp70.1 (c)

a: Oxidative stress induces peroxidation of ω-6 PUFA of the cellular membranes or LDL in the human body, and this leads to generation of the intrinsic hydroxynonenal (HNE). In addition, when vegetable oils containing linoleic acids are used for cooking deep-fried foods, a stable yet very reactive molecule, HNE is generated. In addition, both intrinsic and endogenous HNE induces carbonylation of Hsp70.1 which has the dual functions of a chaperone protein and lysosomal stabilizer. Carbonylated Hsp70.1 is prone to calpain-mediated cleavage and the resultant Hsp70.1 dysfunction provokes lysosomal membrane permeabilization/rupture and aggregation of abnormal proteins, leading to neuronal death.

b: In response to HNE, carbonylation occurs at the key site Arg469 of Hsp70.1 with removal of three amino groups. A decrease of its molecular weight from 157.20 to 113.12 kDa indicates the specific oxidative insult of carbonylation [47].

c: Calpain-mediated Hsp70.1 cleavage occurs *in vitro* in the monkey hippocampal CA1 tissue (5 µg) time-dependently after incubation with 3 mM Ca^{2+} and 2 mM HNE [59]. The cleaved band of ~30 kDa increases with incubation, whereas the main 70 kDa band decreases.

ROS: Reactive Oxygen Species; PUFA: Polyunsaturated Fatty Acid; HNE: Hydroxynonenal; Arg: Arginine Residue, MW: Molecular Weight

In the cortical neurons of the patient with Alzheimer's disease, double-membrane structures presumably indicating permeability of lysosomal membranes, although extremely rare, were found by the high-magnification electron microscopy [33]. Accordingly, the 'calpain-cathepsin hypothesis' which was originally formulated as a mechanism of ischemic neuronal death, can be expanded to the lysosomal theory of neurodegeneration in Alzheimer's disease [32,33,54]. Since there is a casual relationship between hydroxynonenal and degenerative neuronal death, it would be beneficial to scavenge hydroxynonenal to prevent Alzheimer's disease. The lipid peroxidation product, hydroxynonenal is generated during deep-frying of cooking oils containing varying amounts of ω-6 PUFA. After consumption of deep-fried foods cooked with linoleic acids, the level of hydroxynonenal in the bowel and plasma increases so rapidly within minutes to hours and accumulates in the body year by year. Although consumption of ω-6 PUFA-rich vegetable oil has increased worldwide due to its lower cost, unfortunately the adverse effect of vegetable oil-derived hydroxynonenal on health is not widely known [60] (Figures 1a-1c).

Implication of Hydroxynonenal for Alzheimer's Disease

Hydroxynonenal, precisely, 4-hydroxy-2-nonenal, is formed by the peroxidation of ω-6 PUFAs including linoleic and arachidonic acids. Due to the presence of three reactive groups, i.e., an aldehyde (-CHO, also called '-nal'), a double bond at carbon-2 and a hydroxy group (-OH) at carbon-4, hydroxynonenal has powerful oxidative effects on proteins, lipids and genes [61]. It is a highly-reactive carbonyl compound that readily interacts with cysteine, histidine and lysine residues to form protein adducts [62,63]. Because of its lipophilicity, hydroxynonenal can easily permeate cellular membranes to enter the aqueous space of cells and its shuttling occurs within the microsecond range. These characteristics of hydroxynonenal facilitate its reactivity with proteins inside and outside the cell [64].

The common source of hydroxynonenal is an intrinsic one, as it is produced by the peroxidation of membrane lipids. For example, arachidonic acid which is abundant in the gray matter of the brain, can be enzymatically cleaved to generate hydroxynonenal [65-68]. Further, reactive oxygen species attack also lipids of the plasma Low Density Lipoprotein (LDL) to generate hydroxynonenal in the blood [69-71]. In contrast, exogenous hydroxynonenal is generated mainly from linoleic acids during deep-frying of vegetable oils made from rapeseed, soybean, sunflower, etc. (Figure 1a). Surprisingly, after consumption of high-fat diets, the level of hydroxynonenal in the plasma increases significantly within minutes to hours [72]. The most popular fast foods such as fried chicken, fried potatoes, potato chips and fish & chips undoubtedly contain abundant hydroxynonenal. According to the 'calpain-cathepsin hypothesis', activated μ-calpain destabilizes lysosomal membranes by cleaving Hsp70.1 into two fragments (Figure 2a) [30,32,33,39,47,58,60,73,74]. In the postischemic monkey brain, hydroxynonenal causes a specific oxidative injury, 'carbonylation', at the key site Arg469 of Hsp70.1 (Figure 2b) [47,73]. More than ten-fold carbonylated Hsp70.1 was generated in the monkey hippocampal CA1 tissue after transient brain ischemia [47]. Also, generation of large amounts of carbonylated Hsp70.1, especially due to carbonylation of Arg469, was confirmed in the substantia nigra of the same postischemic monkeys [75]. After carbonylation, Hsp70.1 becomes vulnerable to the calpain-mediated cleavage [58,59]. Hsp70.1 cleavage increases *in vitro* time-dependently following hydroxynonenal-mediated carbonylation in the monkey hippocampal CA1 tissue (Figure 2c). Gerónimo-Olvera et al. also demonstrated a similar phenomenon, calpain-mediated degradation of Lamp-2, in the cultured cortical neurons with glucose deprivation/reintroduction (similar to *in-vivo* transient ischemia) [76]. In addition, Xue et al. demonstrated that carbonylation of myofibrillar proteins by oxygen radicals facilitated calpain-mediated cleavage of myosin heavy chain and α-actinin [77]. Taken these data together, it is probable not only in stroke but also in chronic ischemia that activated μ-calpain would induce lysosomal membrane destabilization via cleavage of Hsp70.1, Lamp-2 or myosin heavy chain and α-actinin. Presumably, hydroxynonenal would facilitate cleavage of each protein by carbonylation.

Although a possible link between hydroxynonenal and Alzheimer's disease has already been suggested by many authors until now, the molecular mechanism by which hydroxynonenal causes Alzheimer neuronal death had not been elucidated [50,53,78-83]. However, there are strong experimental, clinical and postmortem evidence suggesting that hydroxynonenal is very closely related to the pathogenesis of Alzheimer's disease. First, it has been shown that hydroxynonenal concentration increases in the brain tissue of the ALDH2*2 transgenic mice which have defective in mitochondrial ALDH2 activity in an age-dependent manner. Such increases were associated with marked neurodegeneration, Alzheimer's disease-like pathological changes and memory impairments [84]. Second, the consecutive but sublethal intravenous injections of the synthetic hydroxynonenal in monkeys caused lysosomal permeabilization/rupture and mitochondrial damages leading to the cell degeneration and death in the hippocampus, hypothalamus and other tissues [60] (Figure 3). Third, elevated levels of hydroxynonenal have been reported in the brain, ventricular fluid and the plasma of patients with Alzheimer's disease [50,78,81] (Figure 1c). Two pathological hallmarks of Alzheimer's disease are extracellular amyloid β plaques and intracellular hyperphosphorylated tau tangles [85-88]. However, the exact roles of these garbage proteins in inducing Alzheimer neuronal death have not been understood until now. Interestingly, high levels of hydroxynonenal were found binding with amyloid β plaques and neurofibrillary tangles [50,80,89,90]. It is therefore tempting and reasonable to speculate from these data that hydroxynonenal bound to amyloid β or tau facilitates carbonylation of Hsp70.1 in the patient brain at the prodromal stage. In this sense, amyloid β or tau is not a direct cause of Alzheimer's disease, but merely a facilitator as an intracerebral source of oxidization. The author speculates that the adverse effects of amyloid β depends mainly on the hydroxynonenal scavenging capacity (i.e., ALDH2 and apolipoprotein E inheritance) of each individual, so the extent of amyloid β deposition does not always correlates with that of dementia [5,6].

Strategy to Scavenge Intrinsic Hydroxynonenal by Aldh2 Activators

The ability of hydroxynonenal to form adducts with proteins is its major deleterious property. The detrimental effect of hydroxynonenal is highly dependent on its concentration and the duration of exposure. When cells are exposed to low dose hydroxynonenal, it can be removed by scavenging and cell viability is not compromised. At exposure to high dose hydroxynonenal, the response will depend on the capacity of cells to eliminate it by scavenging [19]. The key enzymes involved in hydroxynonenal metabolism are Glutathione-S-Transferase (GST), aldo-keto reductase (Akr) and ALDH. GST has a major role for scavenging hydroxynonenal and the conjugation of hydroxynonenal with glutathione by GST is the main step. However, compared to the healthy controls, a decrease in GST activity is observed in all brain areas affected by Alzheimer's disease. Especially, significant decreases are seen in the amygdala, hippocampus and parahippocampal gyrus, inferior parietal lobule and nucleus basalis of Meynert [91]. The reduction
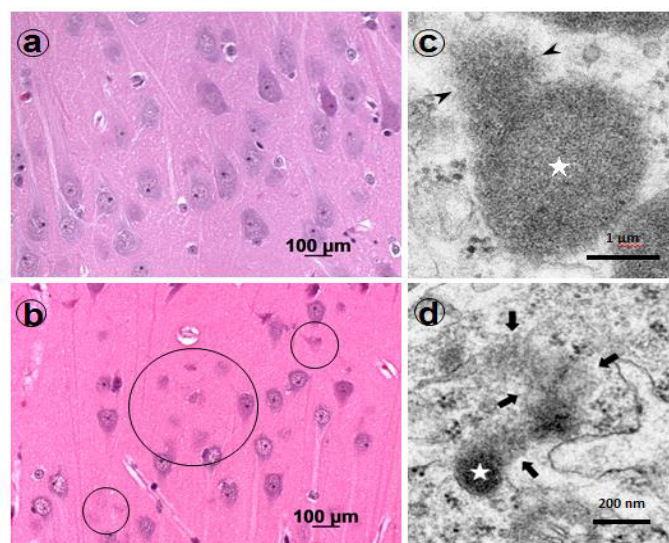
Figure 3: Hippocampal CA1 neuronal death (a,b) caused by lysosomal rupture (c,d)

a: Hippocampal CA1 neurons of the non-ischemic monkey as control.

b: Hippocampal CA1 neuronal death (circles) after HNE injections (5 mg/week x 6 months).

c: Ultrastructural evidence of the lysosomal rupture (arrow heads) in the CA1 neuron of the HNE-injected monkey. Note the poorly defined limiting membrane and numerous internal vesicles characteristic to the lysosome (white star).

d: Spillage of the lysosomal content (arrows) from the lysosome (star) into the degenerating CA1 neuronal perikarya after HNE injections. Conceivably, lysosomes show shrinkage after the spillage.

pathway mediated by the AKR family is thought to be activated in case of acute stress, while the oxidation pathway mediated by ALDH is supposed to be activated when stress is moderate. As ALDH has a prominent role, its down regulation may cause neurodegeneration also in Parkinson disease [92].

Mitochondrial ALDH2 is one of the critical enzymes scavenging aldehyde present in the brain. Interestingly, ALDH2 gene knock-out mice showed accumulation of hydroxynonenal which facilitated accumulations of amyloid β and phosphorylated tau as well as cleaved caspases 3 and 6 [93]. On the other hand, experimental approaches using either pharmacological activation or genetic overexpression of ALDH2 have shown that improved scavenging of hydroxynonenal is protective against both acute (i.e., ischemia) and chronic (i.e., heart failure) cardiovascular diseases [19]. Importantly, ALDH2 is widely expressed in the frontal and temporal cortex, hippocampus, midbrain, basal ganglia and cerebellum [94-96]. A dramatic reduction of the ALDH2 catalytic activity or ALDH2 deficiency, is caused by a structural polymorphism at amino acid position 487, a substitution of lysine for glutamic acid at this position (E487K mutation). A case-control study from Japan found that ALDH2 point mutation is closely associated with occurrence of late-onset Alzheimer's disease, interacting synergically with the presence of the apolipoprotein E allele 4 [97-99]. Further, meta-analysis showed that the variant ALDH2 genotype (GA/AA) significantly increases the risk of Alzheimer's disease, especially in East Asians [100]. The use of a pharmacological ALDH2 activator may therefore prevent neuronal death by decreasing hydroxynonenal-adducts. As no pharmacological therapies have been demonstrated effective for Alzheimer´s disease during the past two decades, the ALDH2 activators have become a notorious focus for the evaluation of new nutraceutical products. ALDH2 is a mitochondrial enzyme which is ubiquitously expressed in all tissues, with a particularly high presence in the liver and brain, where it is mostly involved in the scavenging not only of ethanol-derived acetaldehyde but also of ω-6 PUFA-derived hydroxynonenal [19,101].

Aldehyde Dehydrogenase Activators (ALDAs), represented by Alda-1 (N-(1,3-Benzodioxol-5-ylmethyl)-2,6-dichlorobenzamide), can activate ALDH2 to scavenge hydroxynonenal [22] (Figure 4a). Alda-1 was discovered by Chen et al. with high throughput screening for ALDH2-specific agonists. It is a new class of small molecule activator that directly enhances the catalytic activity of the ALDH2 enzyme and also protects the enzyme against inactivation by adduct formation with its related aldehyde substrates (Figure 4b,4c) [22]. Long-term treatment with Alda-1 attenuates damages by cerebral infarction and also by myocardial infarction (Figure 4d) [102,103]. Further, Stachowic et al. demonstrated that ALDH2 activation by Alda-1 ameliorated hepatic steatosis in apolipoprotein E gene-knockout mice by attenuating formation of hydroxynonenal-protein adducts and decreasing triglyceride content in the liver tissue [104]. During surgery for transplantation of organs such as the liver, the graft organ is subjected to ischemia/reperfusion injury by both hypoxia during a graft's cold storage and reactive oxygen species during restoration of blood flow after transplantation [105]. ALDH2 has been reported to promote cytoprotective mechanisms during ischemia/reperfusion injury by active removal of toxic sub-products of oxidation such as hydroxynonenal [101]. By cleansing the hydroxynonenal and its toxic adducts, ALDH2 helps reduce the vulnerability of the liver graft to ischemic insult and improves protection against the subsequent reperfusion damage, which both lead to a favorable graft outcome after liver transplantation [105]. Accordingly, it was suggested that ALDH2 activators such as Alda-1 have strong potential for use as additives to improve preservative solutions for the protection of graft organ against ischemia/reperfusion injury.

The protective effects of ALDH2 activation on the complex physiopathology of the ischemia/reperfusion injury have been widely confirmed in the brain, heart, intestine and liver [22,102,106-108]. It is therefore possible that diminishing hydroxynonenal accumulation with ALDH2 activators during the prodromal period of Alzheimer's
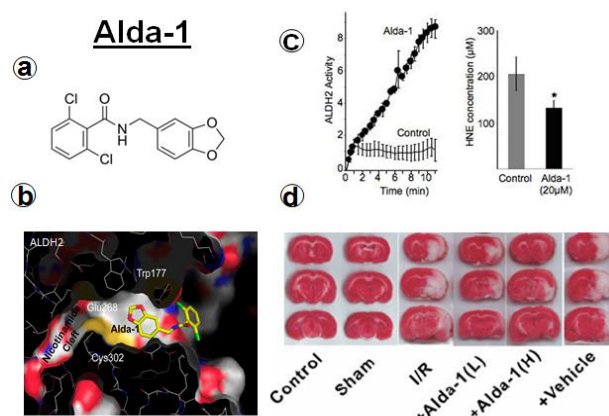
Figure 4: Alda-1 (a), an allosteric agonist of ALDH2 (b), scavenges HNE (c) and attenuates ischemic brain injury in rat stroke models (d)

a: Chemical structure of Alda-1.

b: Crystal structure of Alda-1 in the catalytic tunnel of ALDH2 (Protein data bank accession code PDB: 3INJ). The critical amino acids in this interaction are highlighted [19].

c: Effects of Alda-1 on ALDH2 activation (left) and HNE scavenging (right) [22].

d: Long-term treatment with Alda-1 attenuates damages due to cerebral ischemia/reperfusion. The images show triphenyltetrazolium chloride-stained brain tissue from the control, sham-operated (Sham), ischemia/reperfusion (I/R) and treated with low (L) and high (H) doses of Alda-1, or vehicle. Alda-1 shows a significant reduction of the infarcted (unstained) area, compared to I/R and vehicle treatment [102]. It is likely that the same may occur for preventing neuronal death in Alzheimer's disease.

disease contributes to the reduction or prevention of the disease progression. Epidemiological observation indicates that long-term oral administration of NSAIDs such as flurbiprofen to patients with rheumatoid arthritis, results in reduced risk and delayed onset of Alzheimer's disease. Intriguingly, Hosoi et al. demonstrated that flurbiprofen can increase ALDH2 activity to attenuate protein aggregation and exhibits chaperone activity by assisting protein folding [26] (Figure 5a,b,c). These effects were not achieved simply through its role as an NSAID to inhibit cyclooxygenase activity, instead Hosoi et al. identified ALDH2 as a specific target of flurbiprofen. Flurbiprofen appears to be safe, because it has been used for decades to treat rheumatism, analgesia and inflammatory disorders [26].

Strategy to Scavenge Exogenous Hydroxynonenal by Bacterial ALDH

To protect the lysosomal stabilizer Hsp70.1, the most crucial objective is to scavenge not only intrinsic but also exogenous hydroxynonenal. The latter may be incorporated into the body by deep-fried foods cooked with linoleic acid-rich vegetable oils. Acetic acid bacteria such as *Acetobacter aceti* or *A. pasteurianus* are not a human pathogen but a benign microorganism, present in alcoholic ecological niches such as fruits, flowers and honey bees [109-111]. Since discovery by Louis Pasteur in 1864, they have a long history of safe use in the fermentation industry for the production of vinegar from alcohol. Now adays, acetic acid bacteria are commonly found in naturally fermented foods like wine, vinegar, lambic beer, cocoa, water kefir, kombucha, etc. [112,113]. *A. aceti* or *A. pasteurianus* is a Gram-negative aerobic bacterium with ellipsoidal to rod shaped morphology. It uses sugars and alcohols for its carbon source and turns them into sugar acids or acetic acid. It will grow in any environment where sugar starts to ferment such as wine, beer or sake. The optimum pH for its growth is 4.0 to 6.0 and it grows well at ambient temperature with an optimum between 25~35°C. If water is reduced or removed, cellular activity is decreased [109-111].

Acetic acid bacteria have two membrane-bound enzymes, Alcohol Dehydrogenase (ADH) and Aldehyde Dehydrogenase (ALDH) to convert alcohol via acetaldehyde to acetic acid. Purification and characterization of ALDH from *A. aceti* (recently renamed as *A. pasteruranius*), a practical vinegar fermenter, was first done by Ameyama et al. in 1981 [110]. This enzyme is composed of three subunits: A catalytic subunit of 78 kDa, a cytochrome subunit of 45 kDa and an additional small subunit of 14 kDa [110]. Since ALDH, together with ADH, of acetic acid bacteria converts alcohol and acetaldehyde to acetic acid *in vitro*, it may work also in the bowel to catalyze both of these conversion reactions. Accordingly, nowadays it is used as a supplementat in Japan for preventing hangover occurring after consuming alcohol. It is well-known that human ALDH2 can scavenge not only acetaldehyde but also hydroxynonenal [24,101]. The author therefore conceived the idea that supplementation of *A. aceti* and other appropriate acetic acid bacteria would be useful for catalyzing the conversion of hydroxynonenal in the bowel and attenuating its uptake into the blood (Figure 6).

The fine chemical company Kewpie Corporation, Japan (https://www.kewpie.com/en/), provides a variety of food, cosmetics and pharmaceutical products. This company first isolated abundant amount of acetic acid bacteria during the research for producing high-quality vinegar necessary for their main product, mayonnaise. Recently, Kewpie succeeded in extracting acetic acid bacteria, including more than 10 genera such as *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* etc. from the cloudy vinegar. ALDH from acetic acid bacteria shows a distinct localization (membrane) with human ALDH2 (mitochondria) and ALDH1 (cytosol). ADH and ALDH from acetic acid bacteria are localized at the outer surface of the cytoplasmic membrane and directly oxidize ethanol to acetaldehyde and acetaldehyde to acetic acid, respectively, in the extracellular milleu (for example, in the bowel) [110,111]. When 1 mg of *A. aceti* was incubated with the synthetic hydroxynonenal *in vitro*, 40 nM of hydroxynonenal was oxidized within 1 min. Each enzymatic reaction occurs without NAD or NADP, but proceeds by the electron transport to the ethanol
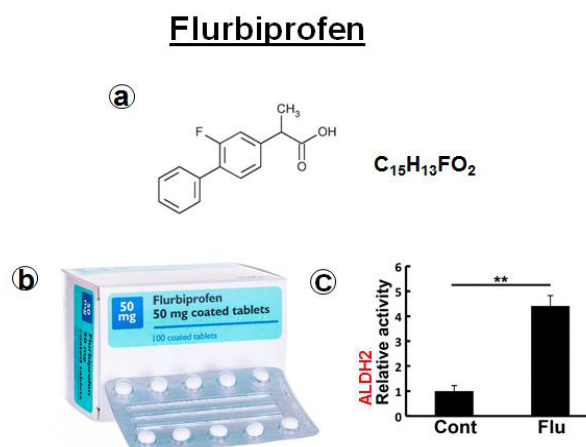
Figure 5: Pharmacological effects of a representative NSAID (a, b) as an ALDH2 activator (c)

a: Chemical structure of flurbiprofen.

b: Analgesic agent 'flurbiprofen' for rheumatoid arthritis.

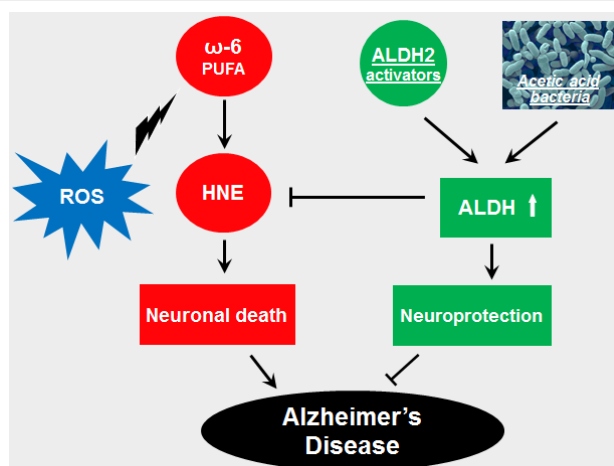c: Flurbiprofen significantly activates ALDH2 [26].

Figure 6: Flow-chart showing progression and prevention of Alzheimer's disease. As HNE causes neurodegeneration via lysosomal rupture, ALDH2 activators or acetic acid bacteria would contribute to neuroprotection and prevention of Alzheimer's disease by scavenging intrinsic or exogenous HNE.

ROS: Reactive Oxygen Species, PUFA: Polyunsaturated Fatty Acid

oxidation respiratory chain which is localized at the membrane [111]. Accordingly, the acetic acid bacteria of Kewpie can function in the absence of coenzymes. Since not only the amount of the ALDH enzyme but also its properties are different from species to species of acetic acid bacteria, a mixture of the genera or species necessary for the vinegar production strengthens its catalytic functions. Intake of two tablets (brand name: *Yoi-toki*) containing 100,000,000 acetic acid bacteria of diverse species prior to consumption of alcohol, prevents hangover by lowering ethanol-derived acetaldehyde. The same may therefore occur for linoleic acid-derived hydroxynonenal to decrease its uptake after eating deep-fried foods.

Mangroves, hosting a broad range of microbes and genes of biotechnological interest, are well-known to possess remarkable abilities to rapidly remove a wide range of pollutants from urban run-off waters. Diverse microbes play an important role in nutrient cycling in mangrove soils, however, studies on the mangrove soil microbiome are scarce [114]. N-ZYME® Group ISO Solution Co., LTD., Thailand & Japan (https://n-zyme.jp/en/), identified a number of previously undiscovered bacteria strains and rare yeasts in the mangrove soil of the gulf of Bangkok. Vacuum-freeze drying was employed to put these bacteria and yeasts into a stabilized product form. N-Zymes are enzyme cocktails which can rapidly degrade and fully digest non-living organic matter and neutralize toxins and they display anti-microbial and anti-fungal properties. They contain several beneficial microorganisms such as *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantalum*, *Bacillus amyloliqufaciens*, *Pichia farinosa*, *Dekkera anomala* and *A. aceti*. Other than diverse enzyme actions, N-Zymes were demonstrated to possess the 3.70~4.08 U/g Piqone enzyme activity of ALDH by the continuous spectrophotometric rate determination (one unit will oxidize 1.0 μM of acetaldehyde to acetic acid per minute at pH 5.5 at 32°C in the presence of β-NAD, potassium and thiols). The daily intake of two tablets of N-Zymes (brand name: *KISLip®*) may contribute to lowering not only acetaldehyde but also hydroxynonenal of exogenous origin. Because of the higher molecular weight and its localization at the bacterial membrane, incorporation of ALDH

from the bowel into the blood is less likely. Nevertheless, catalysis of hydroxynonenal within the bowel would help attenuate its uptake and decrease its level in plasma.

In summary, ALDH2 activators in the blood would help scavenge intrinsic hydroxynonenal, while acetic acid bacteria would help attenuate uptake of the vegetable oil-derived, exogenous hydroxynonenal from the bowel.

Conclusions

Major pharmaceutical industries have failed to develop new, efficacious drugs to control Alzheimer's disease by making too much of the 'amyloid β hypothesis' than necessary. It is therefore a matter of great urgency to re-direct our attention to nutraceuticals and novel bioactive compounds to scavenge its real culprit, 'hydroxynonenal', based on the 'calpain-cathepsin hypothesis'.

By covalently modifying Hsp70.1, both the membrane lipid-(intrinsic) and dietary ω-6 PUFA-(exogenous) peroxidation products 'hydroxynonenal' conceivably play sinister roles in causing neuronal death in Alzheimer's disease. Thus, the unique pharmacological effect of the novel drug Alda-1 or the classic NSAID flurbiprofen to increase ALDH2 activity, as well as acetic acid bacteria containing the ALDH enzyme itself, may contribute to the prevention of Alzheimer's disease by helping scavenge intrinsic or exogenous 'hydroxynonenal', respectively. These may provide a potential therapeutic avenue to reduce the pathology and burden associated with Alzheimer's disease in the world's aging population.

The high serum level of 'hydroxynonenal' found especially in the subjects with ALDH2*2 and apolipoprotein E allele 4 genotypes, would be the most reliable marker for detecting prodromal Alzheimer's disease [54,97-100]. Whether and how the novel ALDH2 activators or acetic acid bacteria will slow down the progression of Alzheimer's disease or actually prevent it in these subjects, will be emergent areas of interests for both the clinical research and pharmaceutical development.

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