

Alzheimer Disease: Mercury as pathogenetic factor and apolipoprotein E as a moderator

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Abstract

The etiology of most cases of Alzheimer's disease (AD) is as yet unknown. Epidemiological studies suggest that environmental factors may be involved beside genetic risk factors. Some studies have shown higher mercury concentrations in brains of deceased and in blood of living patients with Alzheimer's disease. Experimental studies have found that even smallest amounts of mercury but no other metals in low concentrations were able to cause all nerve cell changes, which are typical for Alzheimer's disease. The most important genetic risk factor for sporadic Alzheimer's disease is the presence of the apolipoprotein Ee4 allele whereas the apolipoprotein Ee2 allele reduces the risk of developing Alzheimer's disease. Some investigators have suggested that apolipoprotein Ee4 has a reduced ability to bind metals like mercury and therefore explain the higher risk for Alzheimer's disease. Therapeutic approaches embrace pharmaceuticals which bind metals in the brain of patients with Alzheimer's disease. In sum, both the findings from epidemiological and demographical studies, the frequency of amalgam application in industrialized countries, clinical studies, experimental studies and the dental state of AD patients in comparison to controls suggest a decisive role for inorganic mercury in the etiology of AD.

Abbreviations

ApoE	Apolipoprotein E
AD	Alzheimer's disease
Hg	mercury
GS	Glutamine Synthetase
CS	Creatininkinase
Sulfhydryl group	SH

INTRODUCTION

Alzheimer's disease (AD) rarely occurs in early forms between the age of 30 and 65 (5–10%), and frequently in late forms above the age of 65. On average the duration of the disease is 6 to 10 years, although duration of survival decreases with increasing age. In the US, Alzheimer's disease causes costs amounting to an estimated 90 billion dollars [1]. It ranks fourth among all death causes, meanwhile infesting 4.5 million citizens [2]. According to estimations, a total of 16 million individuals will be affected by the year 2050 [2,3]. In recent years, the incidence of Alzheimer's disease has been on the rise. At least 30–50% of all individuals above the age of 85 are affected in industrialized countries [4]. With ever increasing life-spans Alzheimer's disease will be one of the major public health problems of coming decades.

The central pathogenetic mechanism is neurodegeneration and inflammatory processes, which in turn produce oxidative stress that accelerates neuron damage. The neuro-degeneration starts with a hyperphosphorylation of the tau-protein due to as yet unknown reasons. This in turn leads to a break-down of microtubules which form the cytoskeleton of the neuron and are essential for the neuron's metabolism and functioning. The vital processes of the neuron are disturbed and finally neuron death ensues.

The deposits cannot be adequately removed and form neurofibrillary tangles which in turn accelerate the inflammatory cascade and the positive feedback circle that leads to the progression of the disease. Nerve cell degeneration produces damages in the cholinergic projective systems of the basal prefrontal brain, in the entorhinal cortex, and the hippocampus at early stages [5–7]. The neuronal losses are highest in the nucleus basalis Meynert (Nbm) and reach more than 90% at advanced affection stages [8,9]. Due to the concomitant reduction of the cholinergic activity of the cerebrum, which normally determines the activity status of the cortex, memory performance is significantly impaired despite the fact that the cerebral cortex does not show much damage [5–7]. In the course of Alzheimer's disease, considerable and unusual amounts of extra cellular protein accretions are traceable. Fiber mass consists of insoluble β -Amyloid-Protein ($A\beta$). Therefore, it cannot be removed by antibodies. Accretion of $A\beta$ causes induction of inflammatory processes and an increased creation of free oxygen radicals which are further enhanced through elevated homocysteine and metals [10–14]. This might explain the neurotoxicity of amyloid accretions.

The cause of Alzheimer's disease is yet unknown. About 3–5% of all cases are genetically determined,

suggesting a multi-causal model for the disease. Studies on migration suggest that exogenous factors might be responsible for triggering this pathological positive feedback circle [15–18]. The amount of neurofibrillary tangles found in eminently affected brain regions in Alzheimer's disease correlates with the severity of Alzheimer's disease (Figure 1) [19–22]. Minor neurofibrillary nerve cell changes may occur as early as 50 years before onset of clinical symptoms [19]. Thus, age is not the cause, but only one factor for its clinical manifestation [20]. Interestingly, neurofibrillary tangles in low amounts are already found in about 20% of individuals aged 20–30 years without clinical symptoms of Alzheimer's disease (Figure 1) [20]. In the age group 70–80 yrs., 90% of the individuals display neurofibrillary tangles in their brains. In this cohort, 35% have highest numbers of histological detectable neurofibrillary tangles and subsequently suffer clinically detectable from Alzheimer's disease (Figure 2) [20]. Thus, if an exogenous factor contributes to the development of neurofibrillary tangles and consequently Alzheimer's disease, this factor must be present in a great portion of the public only in industrial developed countries. In the past 20 years, a number of studies were published suggesting a potential pathogenetic role of inorganic mercury in Alzheimer's disease. In this article, we performed a multidisciplinary review of the material published so far.

SEARCH STRATEGY

The data base Medline was searched using Ovid Technologies, Version rel9.1.0 for 1966–16.1.2004 with the keywords (mercur\$) and (neurotoxic\$ or alzheimer\$ or dement\$). This search was supplemented from the bibliography of retrieved articles. Also, we searched the internet using Google. Additionally, the current knowledge about Alzheimer's disease was screened in literature about neurology. We tried to come to a fair assessment of the situation by a multidisciplinary review of the material by several researchers with different leanings and preconceptions, and by discussing difficult findings.

APOLIPOPROTEIN E

A well known genetic risk factor for the early and late forms of Alzheimer's disease is the polymorphism of the apolipoprotein E gene (APOE). APOE occurs in three genetic variants which are determined on chromosome 19: APOE2, APOE3 and APOE4 [23]. Apolipoprotein E is a lipid transport protein regulating uptake and excretion of lipids, and it is normally only considered in this capacity [23]. Interestingly, high concentrations of apolipoprotein E are found in the central nervous system where apolipoprotein E is expressed in astrocytes [24–26] where it may play an important role in the distribution of cholesterol and phospholipids [27]. Presence of the APOEe4 allele increases the risk of developing Alzheimer's disease by reducing the average age of disease manifestation [28]. On the other

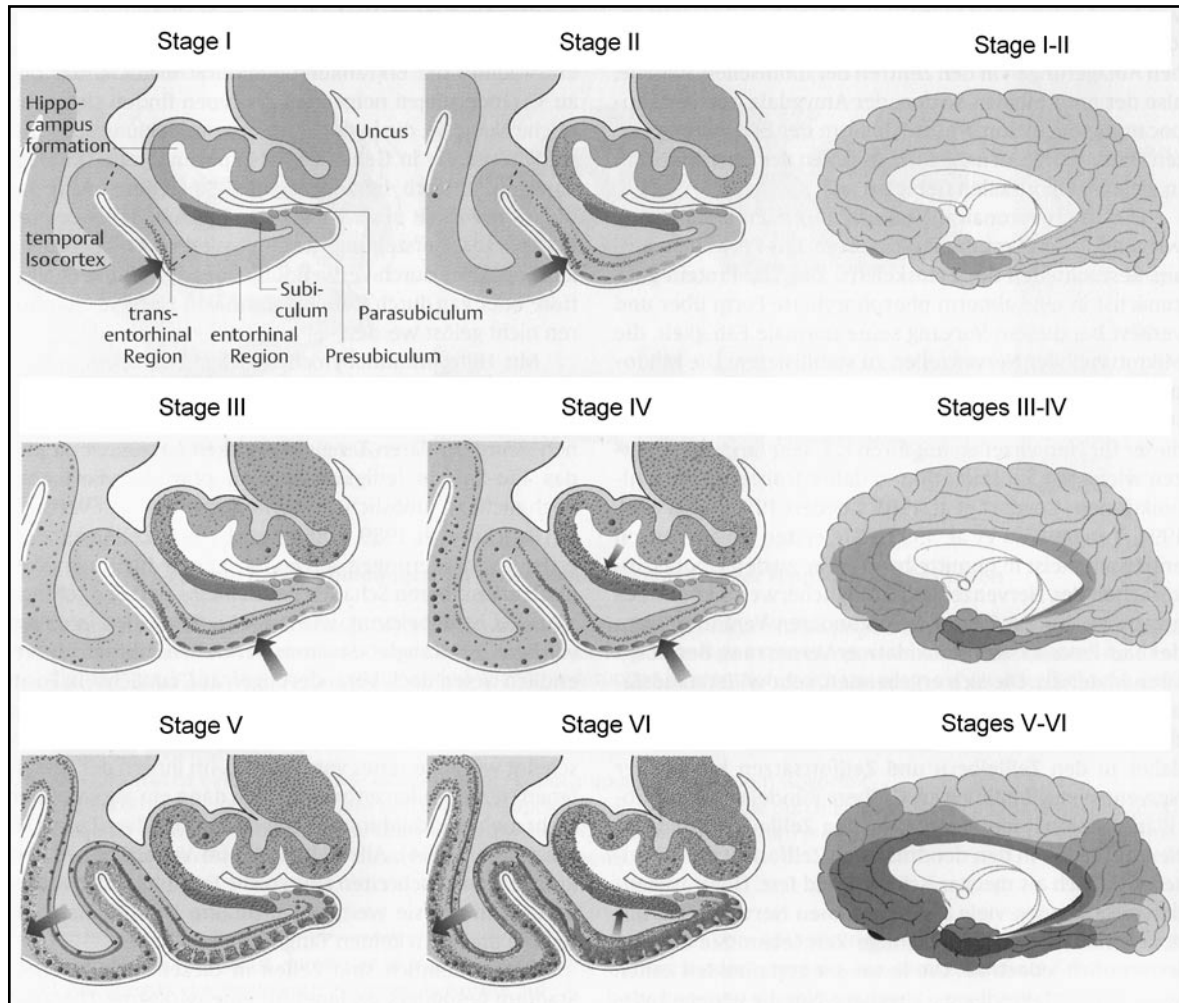


Figure 1. Distribution pattern of Alzheimer's disease-related neurofibrillary tangles

Frontal sections of brain samples with typical Alzheimer's disease-related neurofibrillary tangles (NFT). The left and middle part of the figure show the uncus and the anterior part of the gyrus parahippocampalis. The distribution pattern and the severity of the lesions permit distinction of six NFT stages. The first two stages (I and II) do not yet show clinical symptoms. Stage III is associated with initial cognitive deficits which establish the diagnosis. The severe stages V and VI are associated with the full-blown clinical picture of Alzheimer's disease. The arrows mark key neuropathologic features typical for the different stages. The right part of the figure shows the progression of Alzheimer's disease-related lesions in a right hemisphere (seen from medial)(acc. to Braak et al. ²⁰).

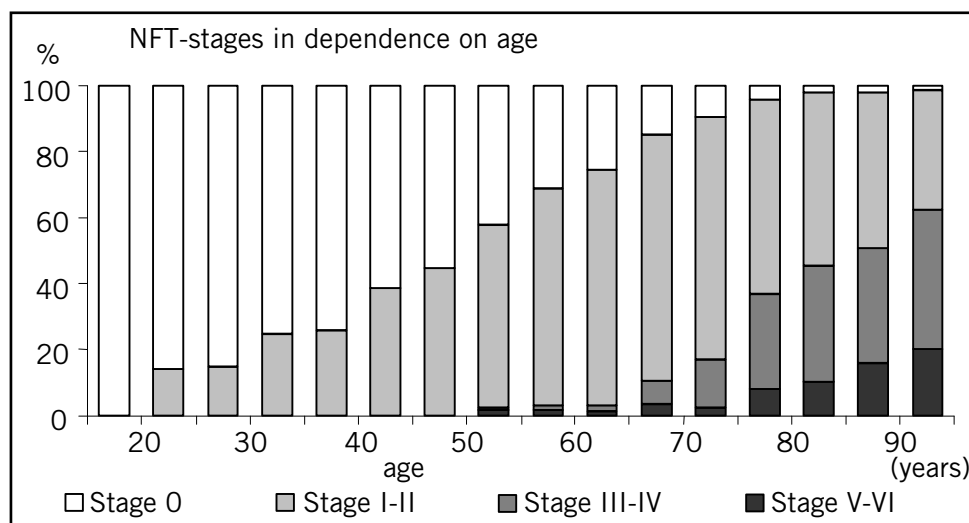


Figure 2. Frequency of stages (I through VI) according severity of neurofibrillary tangles (NFT) in brain samples depending on age (n=3261)

Frequency of stages of Alzheimer-related lesions in different age categories: the diagram represents a total of 3261 autopsy cases. White columns represent cases without neurofibrillary tangles (NFT). Pale-grey sections correspond to stages I and II, dark-grey sections to stages III and IV, black sections to stages V and VI (also see Figure 1) (acc. to Braak et al. ²⁰)

hand, presence of the APOE ϵ 2 allele appears to reduce affection risk [28]. A meta-analysis with 6000 patients and 8000 controls showed that APOE ϵ 4/ ϵ 4 homozygotes have a relative risk of developing Alzheimer's disease of 14.9 compared with APOE ϵ 3/ ϵ 3 homozygotes, whereas combinations with APOE ϵ 2 are protective (Table I) [29].

Apolipoprotein E consists of 299 amino acids. At position 112 and 158, different amino acids occur: Apolipoprotein E2 contains 2 cysteines, apolipoprotein E3 contains 1 cysteine and 1 arginine, and apolipoprotein E4 contains 2 arginines [23]. In contrast to arginine, cysteine contains one sulfhydryl group (SH), at which metals may bind, especially chemically bivalent metals (e.g. lead, mercury, copper, zinc). Therefore, some variants of apolipoprotein E, namely those showing cysteine rests (i.e. apolipoprotein E2 and E3), could bind and detoxify heavy metals in the nerve cell and liquor, while others (apolipoprotein E4) cannot [30].

Recent observations may confirm this assumption. 529 individuals who had been in contact with lead 16 years ago, were tested neuropsychologically, and lead content in bones, as well as APOE-type was examined. Persons having at least one APOE ϵ 4 allele had poorer test results than those with the same lead content but without the APOE ϵ 4 allele [31]. In a group displaying symptoms from dental amalgam, 400 participants, as opposed to 426 healthy control, had significantly more frequently the APOE ϵ 4 allele and less often the "protective" APOE constellations (APOE ϵ 2/ ϵ 2 and APOE ϵ 2/ ϵ 3) [32].

It should be noted, however, that presence of the APOE ϵ 4 allele is not a necessary condition for the development of Alzheimer's disease. In over 50% of the patients with Alzheimer's disease, no APOE ϵ 4 allele can be found, and one study was able to demonstrate that 85% of individuals older than 80 years with an APOE ϵ 4/ ϵ 4 status did not suffer from cognitive impairments [33]. African populations show a frequency of APOE ϵ 4 allele of up to 40% (Europe 15%) but are nonetheless affected to a lower degree than populations of Western industrial countries [34]; whether this is due to less efficient diagnostic facilities or a true difference remains to be seen. Conversely, Afro-Americans show a significantly increased risk of Alzheimer's disease than Caucasians [35].

MERCURY AND ALZHEIMER'S DISEASE

Major human sources of mercury include fish consumption [36, 37], dental amalgams [38] and vaccines [36]. Regular fish consumption and intake of Omega-3-fatty acids reduces the risk of developing Alzheimer's disease [35,39–42]. Selenium, which is found in fish, is essential for the function of glutathione peroxidase regenerating glutathione, which is a important antioxidant and detoxification enzyme. Furthermore, selenium disposes mercury directly by tightly binding it to mercury selenite, which is non-toxic. This is shown by autopsy studies that assess the ratio of selenium and mercury in several organs [43]. Methyl mercury

in fish, being bound to cysteine, appears to be by far less toxic than hitherto assumed and is about 20 times less toxic than methyl mercury chloride usually used in experiments [44]. For that reasons, methyl-mercury found in fish seems not to be involved in the pathogenesis of Alzheimer's disease. Inorganic mercury (found in dental amalgam) or ethyl-mercury (found in vaccines) may play a major role.

Experimental mercury effects and Alzheimer's disease

Inhibition and deterioration of neurotubulin

It was shown that both organic [45] and inorganic mercury [46] cause those biochemical changes in tubuli structures which can be found in brains of patients with Alzheimer's disease [46]. In healthy human brain tissue cultures, only mercury, even in lower concentrations, but not aluminum, lead, zinc or iron were able to inhibit binding to guanosine-tri-phosphate (GTP), which is necessary for tubulin synthesis and thus for neuron function [46]. Mercury inhibits ADP-ribosylation of tubulin and actin [47]. This process leads to an inhibition of polymerization of tubulin to microtubulin. As a result, neurofibrillary tangles and senile plaques are formed. Living rats exposed to mercury vapor (250+300 μ g/m³) four times a day exhibit the same molecular changes in their brain tissue as those caused in human brain cell cultures after 14 days [30]. These changes are similar to those found post mortem in brains of patients with Alzheimer's disease [46, 48, 49]. Tubulin is assumed to be the most vulnerable protein for mercury, because administration of very low doses of inorganic mercury do not inhibit other GTP- or ATP-binding proteins [48, 48]. Tubulin has at least 14 sulfhydryl groups which bind mercury with high affinity resulting in functional losses of tubulin and creation of neurofibrillary tangles. Since human nerve cells do not regenerate, any blocking of neurotubulin is particularly grave.

Creation of neurofibrillary tangles and amyloid

Administration of very low doses of inorganic mercury (0.18 μ M) has been shown to promote hyperphosphorylation of tau-protein in neuronal cell cultures within 24 hours [50]. Hyperphosphorylation of tau is the first biochemical change to be observed in the development of Alzheimer's disease and results in formation of neurofibrillary tangles and failure of nerve cell functions. Administration of mercury to nerve cells provokes also production of β -amyloid 40 and 42 [50].

Glutathione consumption and increased oxidative stress

Within 30 minutes, low doses of inorganic mercury reduce glutathione concentration by increasing oxidative stress in a cell culture model. Addition of melatonin is able to protect the nerve cells from the damaging impact of mercury [50]. Melatonin is an antioxidant and in addition has the ability to bind and eliminate metals [51]. Although cobalt also has been

Table I. Alzheimer's disease as a function of the APOE genotype

Relative Risk	APOE Genotype	Pct of US Population	Diagnosis at Age	Sulfhydryl-Groups (SH)
0.6	2/2	< 1	?	4
0.6	2/3	11	> 90	3
1.0	3/3	60	80–90	2
2.6	2/4	5	80–90	2
3.2	3/4	21	70–80	1
14.9	4/4	2	< 70	0

Table II. Overview of the effects of mercury found by in vitro studies and animal experiments

Effect	References
Hyperphosphorylation of the tau-protein	[50]
Formation of neurofibrillary tangles in nerve cells	[54, 50]
Cessation of tubules function through impairment of nucleotides	[30, 46, 47, 48, 49]
Increased production of amyloid β -protein	[50, 52]
Enhanced oxidative distress	[50, 52]
Degeneration of nerve cells	[54]
Reduction of the amount of glutathione (GSH)	[50, 52]
Binding of Selenium and reduction of available selenium	[44]

reported to decrease glutathione concentration in neuronal cell cultures and to release secretion of β -amyloid [52], it is not able to hyperphosphorylate tau-protein and built up neurofibrillary tangles [53]. Changes brought about by Cobalt were only observable above concentrations 1700 higher than those of mercury (300 μ M Cobalt versus 180 nM mercury) [52,53].

Neurodegeneration through mercury

Leong et al. [54] demonstrated axon degeneration and formation of neurofibrillary tangles in animal neuronal cell cultures within minutes and with lowest amounts of inorganic mercury (2 μ l 100 nM in 2 ml neuronal cell culture nourishing solution). This neurodegenerative effect was not demonstrable with other metals like aluminum, lead, cadmium, or manganese [54]. In neuronal stem cells, inorganic mercury of 2 and 5 μ g/ml impaired tubulin functions for 48 hours [55]. It caused apoptosis, the programmed cell death of nerve cells, and induced expression of heat shock proteins [55].

Comparison with mercury concentrations in human brain tissues

Mercury load in the brain of patients with Alzheimer's disease was specified at 20 and 178 ng/g [56–58]. This amounts to a molar mercury concentration of 0.1 to 0.89 μ Mol. In the above mentioned experimental studies on nerve cells, exclusive administration of mercury of a final concentration of 0.0001 μ Mol (2 μ l 0.1 μ Molar mercury in 2ml nourishing solution) resulted in axon degeneration and creation of neurofibrillary tangles [54]. Addition of 0.18 μ Mol mercury lead to secretion of β -amyloid 40 and 42, to increased oxidative stress and to hyperphosphorylation of the tau protein [50,52].

Increase of glutamate toxicity

It is assumed that toxicity of the excitatory neurotransmitter glutamate plays a role in neuronal death in neurodegenerative diseases [59]. Glutamate is toxic when it accumulates and when protective mechanisms fail. One such protective mechanism is the enzyme glutamine synthetase primarily found in astrocytes [60]. Mercury inhibits re-uptake of glutamate in the astrocytes and other cells of the nervous system [61,62] resulting in extracellular accumulation of glutamate. In addition, mercury and lead inhibit the enzyme glutamine synthetase which converts glutamate to nontoxic glutamine [63]. Inorganic mercury (Hg^{++}) appears to inhibit GS to a larger degree than methyl mercury [64]. It has been shown that glutamine synthetase is reduced in the brain of patients with Alzheimer's disease [60,65], whereas the concentration of glutamine synthetase in the liquor is increased [66]. Glutamine synthetase concentration in the liquor, stemming from enhanced degradation of astrocytes having a high concentration of glutamine synthetase, could thus have diagnostic relevance for Alzheimer's disease [66,67].

Enzyme inhibition

Creatininkinase (CS) is an enzyme crucial for energy production in all body cells. Its function is reduced in patients with Alzheimer's disease [68]. Since it possesses many sulfhydryl groups, similar to glutamine synthetase and tubulin, mercury inhibits its functions [18].

With respect to Alzheimer's disease, protein kinase plays an important role in the production of normal amyloid. Whenever these are inhibited, the enzyme β -secretase modulates the metabolisms of APP (Amyloid Precursor Protein) in a way that β -amyloid is increas-

ingly produced [50]. Protein kinase C is inhibited by mercury both in vitro and in the brain tissue [69,70].

Synergistic effects of other metals

The Alzheimer's disease-typical neuronal changes (hyperphosphorylation of tau-protein, occurrence of neurofibrillary tangles, β -amyloid, tubulin inhibition, axon degeneration, increase of glutamine synthetase in the liquor) found in nerve cells and animals may not be caused by other metals (lead, cadmium, aluminum, copper, zinc, iron, chrome, manganese), but other metals may potentize mercury effects by contributing to oxidative stress [18, 46, 53, 54].

Experimental effects of estrogens

Estrogen is able to compensate the damaging effects of mercury in a cell model, when it is concurrently administered [52]. This could provide an explanation for the findings of some studies showing reduced risk of developing Alzheimer's disease with high dose estrogen replacement [52, 71–73], which, however, is not a consistent finding [74].

Summary of experimental mercury effects

In lower doses, mercury has been shown to have biochemical effects on nerve cells, both in in vitro and in animal experiments, which are typical for Alzheimer's disease (Table II):

Mercury in patients with Alzheimer's disease

Mercury in brains of patients with Alzheimer's disease

Ehmann et al. [56] examined 81 brain specimens from 14 patients with Alzheimer's disease and 147 specimens from 28 controls with the same age. Out of 17 target elements, the biggest differences were found for mercury and bromine levels in the cerebral brain tissue of patients with Alzheimer's disease (3.4 ± 3.7 ng/g versus 17.5 ± 1.3 ng/g, $p < 0.05$). In the gray matter, they found more mercury (patients with Alzheimer's disease: 42.7 ng/g versus 14.7 ng/g, controls: 29.0 ng/g versus 20.5) [56]. In the nucleus basalis Meynert, mercury concentration was four times higher in 14 patients with Alzheimer's disease compared with 15 controls [57]. Other elements were significantly increased, too (iron, sodium, and zinc) [57]. In tissue specimens from temporal lobes of 10 patients with Alzheimer's disease and 12 controls, there were significant increases of mercury concentrations in the microsomes of the brain cells and non-significantly increased mercury values in other brain fractions (temporal lobe, mitochondria, and cell nuclei) [75]. The total mercury content in the temporal lobes of the patients with Alzheimer's disease was 176 ng/g, as compared to 69.6 ng/g of the controls [75].

In the pituitary, significant differences were found for mercury content between 43 patients with Alzheimer's disease and 15 controls [76]. Other studies found non-significantly increased mercury values in

the olfactory region of the amygdala [77] and amygdala and hippocampus, respectively [78], but non-significantly decreased mercury values in the cerebellum and rhinencephalon [78]. Another study found no elevated mercury levels in brains of patients with Alzheimer's disease compared to controls [79].

Saxe et al. [58] were unable to find significant mercury increases in an autopsy study of specific brain regions of 68 patients with Alzheimer's disease (mercury on average 20.3 – 61.1 ng/g depending on the area) when compared to 33 controls with the same age (mercury on average 30.1 – 88.9 ng/g). Also, there was neither a correlation between number and duration of amalgam filling and mercury concentration in the Alzheimer's disease group nor in the control group [58]. This is astonishing because other human autopsy studies show such correlations [80–87]. Interestingly, in patients unaffected by Alzheimer's disease, mercury concentration in the olfactory region was twice as high as in controls (88.9 ng/g versus 41.7 ng/g) [58]. In sum, autopsy studies examining mercury load in the brains of patients with Alzheimer's disease, although suggestive, are not consistent. One potential confounding factor might be the loosely defined staging of patients with Alzheimer's disease when autopsied.

Mercury in living patients with Alzheimer's disease

Mercury blood concentration in 33 patients with Alzheimer's disease was twice as high (2.64 μ g/l) as that of 45 depressed patients (1.20 μ g/l) and 65 patients without psychiatric disorders (1.09 μ g/l) ($p < 0.0005$) [88]. In the early form of Alzheimer's disease (13 persons younger than 65 years), difference was even higher (3.32 μ g/l, $p = 0.0002$). Correlation with blood mercury concentration and the amyloid- β -protein ($A\beta$) in the liquor cerebrospinalis was significant ($p = 0.0015$). These findings were confirmed [89], yet disconfirmed [90]. The average mercury load in the urine of 9 patients with Alzheimer's disease (2.96 ± 1.13 μ g/l) as compared to 9 controls (1.86 ± 0.9 μ g/l) was not significantly different [90]. This obviously is a power problem, as the effect size of this difference (standardized mean difference) is 1.1 and thus large.

Several studies measuring the nails and hair of patients with Alzheimer's disease found less mercury in the patients compared to controls [91,92]. This could be attributable to the fact that nail and hair merely reflect a recent mercury exposure. Patients with Alzheimer's disease would be less exposed to environmental mercury than controls because they are accommodated in foster homes [92]. Mercury concentrations in the blood may only be elevated when nerve cell damage is greatest (presumably at intermediate stages), suggesting a curvilinear association between Alzheimer's disease and mercury excretion, a possibility that researchers have eschewed so having normally supposed and looked for a linear relationship.

Dental condition and the risk of developing Alzheimer's disease

A recent analysis of 10,263 individuals from Canada yielded a distinct association between dental condition and the risk for Alzheimer's disease. The fewer the number of teeth the higher the risk [93]. The authors took this as evidence for the fact that amalgam fillings are not causal for Alzheimer's disease [93]. Alzheimer's disease takes about 30–50 years to clinically manifest itself [20]. Patients with fewer teeth previously had poorer dental conditions and had therefore presumably been provided with mercury containing amalgam over longer period. Thus, they are likely to have been exposed to mercury-vapor in a vulnerable phase and to a larger extent than persons still having teeth in advanced years.

Metal chelation as potential therapy of Alzheimer disease?

If mercury is involved in the genesis of Alzheimer's disease, preventive and possibly therapeutic strategies may be developed provided neurodegeneration has not progressed too much.

It has been suggested that aluminum and iron play an important role in Alzheimer's disease [94]. Therefore, the chelator desferrioxamine, which has the capacity to bind iron, aluminum and to a lesser degree mercury was tested in clinical trials [95,96]. Recently, clioquinole, formerly approved as an antibiotic in Japan, has been successfully applied in animal [97] and clinical studies [98–100] to treat Alzheimer's disease due to its capacity as a chelating agent to bind copper and zinc. Chelating agents, which bind copper and zinc usually also have the capacity to bind mercury.

IMPLICATIONS

Converging findings from experimental, epidemiological, and clinical studies identify inorganic mercury as one of the potential exogenous factors responsible for Alzheimer's disease. The main source for inorganic mercury in habitants of industrial developed countries is dental amalgam [38, 101]. Other metals and noxes might have synergistic effects with mercury. Due to the complex relationships and restricted technical measurement equipment in some studies, individual findings appear contradictory and a lot remains to be clarified. This is a situation analogous to other hotly debated areas, like the association of smoking and cancer, or estrogen replacement and myocardial infarction.

Obviously, definite knowledge about the causal role of mercury in Alzheimer's disease may only be derived from large, long-term prospective epidemiological studies examining occurrence of Alzheimer's disease in subjects exposed to the risk of mercury in amalgam and other sources, compared with those at lower risk. Since apolipoprotein E could constitute an important protective or risk factor, it should be monitored in future studies. Additionally, clinical studies with chelating agents measuring mercury excretion could give in-

direct evidence and would offer a therapeutic strategy for Alzheimer's disease at early stages. The findings reviewed here should have made plausible that the potential association between mercury and Alzheimer's disease should be one of prime importance, since the public health impact is enormous.

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