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The Relationship of the Toxic Effects Of Mercury To Exacerbation Of The Medical Condition Classified As Alzheimer's Disease

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Abstract: Mercury in ionic and vapor form when exposed to normal brain tissue is capable of causing the same biochemical aberrancies found in Alzheimer's diseased (AD) brain with the rapid inactivation of tubulin, creatine kinase and glutamine synthetase. Mercury exposure to neurons in culture is also capable of effecting elevated production of amyloid protein, hyper-phosphorylation of Tau and the stripping of tubulin from neurofibrils causing the production of neurofibrillary tangles. I propose that mercury, and other supporting factors, that have more specificity for thiol-sensitive enzymes such as tubulin and creatine kinase are the major environmental toxicants that are the etiological source of AD. This hypothesis is supported by the genetic susceptibility expressed through the APO-E gene family. Here a reduction of APO-E gene types carrying cysteines decreases the ability to remove mercury from the cerebrospinal fluid and increases the risk of AD.

RATIONALE FOR THE HYPOTHESIS

After World War II a long-term study was done on about 500 sets of identical twins to identify which diseases were genetic in nature or cause. AD was identified as primarily not being of genetic origin, but with having a genetic susceptibility component that also required a toxic insult. This insult could be of infectious or chemical toxicant source. However, after thousands, if not millions, of microscopic evaluations of AD brain samples and other significant testing no one has identified an infectious vector for AD. This leaves a chemical toxicant as the most likely causal agent. Observing that the rates of AD is about the same in the different states and city versus country dwellers it is apparent that any toxicant involved would not be in the general environment. Also, a man and woman can live together on a farm, eat the same food daily, sleep in the same house and one will get AD and one will not. This supports the absence of an infectious vector and, most importantly, indicates that the toxic exposure must be very personal and is coupled to a specific genetic susceptibility. It was reported that the heavy metal mercury was elevated in AD versus normal age-matched brain, but no one wanted to conclude that it was causal for AD. When one looks for a toxicant that is widespread and personal we are limited to what we place in our bodies on an individual basis. This specifically limits the exposures to foods, vaccines and dental implants. All three of these can contribute to overall mercury body burden. However, organized dentistry has successfully convinced organized medicine that the level of exposure to mercury from dental amalgams was too low to be toxic. However, recent studies confirm that dental amalgams are the major contributor to mercury body burden. Therefore, we did initial studies to determine if mercury could reproduce any of the aberrant biochemistry observed in AD brain. The studies were very positive and make sense in that the studied proteins that were most affected in AD brain are those that have reactive thiol groups in their structures that are especially sensitive to mercury inhibition. Since the initial studies by our laboratory several other labs have confirmed our results and have added major observations, including positive results on producing elements of the diagnostic hallmarks found in AD brain. While one can argue forever that some doubts remain about mercury exposure being the cause of AD it is obvious that, since mercury inhibits the same enzymes found inhibited in AD brain, exposure to a constant level of

mercury from dental amalgams for 40-50 years would definitely rush the onset of AD and would exacerbate the biochemical factors related to this disease. This is elaborated on in the following paragraphs.

Research regarding Alzheimer's disease (AD) in the research done in our laboratory has been directed towards detecting aberrancy in the nucleotide binding proteins of AD post-mortem brain versus age matched control brain samples. Basic to all of our findings is the following observation. Two very important brain nucleotide binding proteins, tubulin and creatine kinase (CK), show greatly diminished nucleotide binding ability and they are abnormally partitioned into the membrane fraction of AD brain tissue (1,2). It is critical to understand that both tubulin and CK should primarily be found in the soluble fraction of a brain homogenate. However, both are almost totally located in the particulate fraction after separation of the soluble and particulate fractions by simple centrifugation. Yet, both proteins appear of normal size and unmodified on reducing gel electrophoresis. This indicates that both tubulin and CK have formed crosslinks with other proteins that are insoluble and, that these crosslinks are disrupted by reduction before gel electrophoresis. What tubulin and CK have in common is that both have a very reactive sulfhydryl in their enzyme active sites that, if modified, inhibits their biological activity (14, 15). Mercury has a very high affinity for sulfhydryls and has been proven to be a potent inhibitor of both of these proteins biological activity. Also, mercury is divalent and can form crosslinks between soluble proteins like tubulin and CK. For example, $2 \text{ Protein-SH} + \text{Hg}^{2+} \rightarrow \text{Protein-S-Hg-S-Protein} + 2 \text{ H}^+$, forming aggregates that would abnormally appear in the particulate fraction.

Both tubulin and CK are proteins that bind the nucleotides GTP (guanosine-5'-triphosphate) and ATP (adenosine-5'-triphosphate), respectively. We use a "photoaffinity labeling" technology to determine the availability of these binding sites before and after addition of mercury or other toxicants. In this manner our laboratory demonstrated that both tubulin and CK had diminished biological activity in AD brain, and only AD brain. Since AD is not directly a genetically inherited disease we searched for possible toxicants that might mimic the specific findings observed in AD brain. Our first finding was simple and straight-forward. After testing numerous heavy metals we observed that only mercury-II cation (Hg^{2+}) could mimic this effect in homogenates of normal brain at concentrations that might be expected to be found in brain (3,4). The observation was that Hg^{2+} at very low micromolar levels (@ 1 micromolar) could selectively, rapidly and totally abolish the GTP binding activity of tubulin ($M_r = 55,000$ daltons) without any noticeable effect on other GTP or ATP binding proteins ($M_r = 42,000$ daltons) that are also present in both control and AD brain. Therefore, addition of mercury, and only mercury, to control brain homogenates gave a nucleotide binding profile that was identical to that observed in AD brain (4,5,6). Further, recent results in our laboratory have shown that the addition of Hg^{2+} to control brain homogenates not only caused the decrease in nucleotide interaction but could also cause the abnormal partitioning of tubulin into the particulate fraction as observed in AD brain (7). This was especially effective in the presence of other divalent metals, such as zinc, which is elevated in AD brain.

The next set of experiments was to determine if mercury vapor, the form that escapes from dental amalgams, could mimic the effect in rats exposed to such vapor for various periods of time (5). Rats are different from humans in that their cells can synthesize vitamin C whereas humans have to ingest vitamin C. Vitamin C is thought to be somewhat protective against heavy metal toxicity and other oxidative stresses. However, we observed that the tubulin in the brains of rats exposed to mercury vapor lost between

41 and 75 percent of the nucleotide binding capability demonstrating a Hg²⁺ induced similarity to the aberrancy observed in AD brain (5).

Consistent with this was a recent report by Dr. Michael Aschner of Wake Forest University at the 1998 Spring IAOMT meeting. He stated that Western blot analysis of brains of rats exposed to mercury vapor (as above) showed elevated levels of an enzyme called glutamine synthetase (GS) when compared to non-treated controls. This is consistent with a report published from our laboratory in 1992 where we predicted that the elevation of GS in the cerebrospinal fluid of AD patients had potential as a diagnostic marker for AD (12). This potential value of GS as a diagnostic aid for AD was recently confirmed by German scientists (16) and is now being developed by Affinity Labeling Technologies (<<http://www.altcorp.com>>www.altcorp.com) for commercial use.

Even though mercury has inhibitory effects on tubulin, CK and GS and that these proteins are proven to be aberrant in AD, this still does not conclusively prove that mercury exposure causes AD. However, it definitely proves that chronic, daily exposure to mercury would exacerbate the clinical conditions of Alzheimer's disease by the ability of low doses of mercury to inhibit enzymes known to be inhibited in AD brain. Is such an exposure to mercury likely? The answer is yes. Dental amalgams, or silver fillings, are 50% mercury by weight and it is quite easy to demonstrate that mercury vapors readily emit from these filling and this has been confirmed by an NIH study indicating that individuals with an average number of amalgam fillings have about 4.5 times the blood/urine mercury levels as controls without amalgams (20).

We were interested in the genetic susceptibility research regarding AD and followed this work to see if it correlated to our results. That is, does susceptibility to heavy metal toxicity have any relationship to AD? When we read the correlation of APO-E4 to age of onset of AD we were intrigued enough to look at the primary structure of this protein and its alleles, APO-E2 and APO-E3. In general, the story is this. Individuals with APO-E2 or combinations of APO-E2 and E3 are much less likely to get AD than are individuals who have inherited APO-E4 genes. Also, APO-E2 appears to be more protective than APO-E3 against AD. What is the basic structural difference between these three alleles? Simply, the protective APO-E2 has two sulfhydryls (cysteines) that can bind mercury or other heavy metals that APO-E4 lacks. For example, in APO-E3, one of these cysteines is replaced by an arginine and in APO-E4, both of the cysteines are replaced by arginine. Therefore, lack of protection against AD follows loss of sulfhydryls from APO-E proteins (6).

What does the APO-E protein do? It is involved in cholesterol transport and all three forms work reasonably well at this. However, APO-E is classified as a "housekeeping protein". That is, in contrast to tubulin and CK, which are meant to stay inside of cells where they are synthesized, APO-E is meant to leave the brain cells carrying out unwanted material for the body to dispose of. In the brain, APO-E protein leaves the cells and goes into the cerebrospinal fluid (CSF) and then crosses the blood-brain barrier into the blood plasma. It is cleared from the blood by processes that dispose of the unwanted material that it is carrying. It is our hypothesis that while APO-E2 or E3 are leaving the brain cells and traversing the CSF they likely bind and remove any heavy metal or other sulfhydryl reactive toxin that may have made it into the central nervous system (6). APO-E4 could not do this and therefore does not provide the protective parameters that APO-E2 and E3 have. It is interesting to note that the second highest level of APO-E protein in the body is in the CSF that bathes and protects the brain.

There was considerable debate concerning whether or not mercury reaches levels in the brain that could be considered toxic. The determination of the levels of mercury toxicity

that could cause neurological disease has been done using animals, such as rats, under tightly controlled laboratory conditions where the diet is carefully monitored to exclude other toxicants. Also, any rat that becomes ill or infected by microbial sources is removed from the study. However, humans do not live under such restricted conditions. For example, heavy metal imbalances in AD brains have been reported numerous times. Cigarette smokers are exposed to excess cadmium (Cd) and lead (Pb) toxicity is not that uncommon in the inter-city environment or for those exposed to leaded gasoline fumes for many years. The latest research in our laboratory has shown that one can add various metals to human brain homogenates to levels that do not affect nucleotide binding to tubulin yet the very presence of these metal potentiate the toxicity of mercury. That is, the presence of Zn^{2+} and Cd^{2+} , at non-toxic levels, decrease the amount of Hg^{2+} required for 50% inhibition of tubulin or creatine kinase viability. When we compare the toxicity of Hg^{2+} in brain homogenates as described above (refs. 3 & 4) the addition of 0, 10 and 20 micromolar Zn^{2+} increases the inhibition of GTP binding to tubulin from 4% to 50% and 76%, respectively (7,13). In other words, mercury is much more toxic in the presence of other metals that compete with mercury for the binding sites on protective biomolecules (e.g., APO-E2 & E3, glutathione, metallo-thionine, etc.). This observation probably explains some observations on the toxicity of solutions in which dental amalgams have been soaked.

Through the same rationale, illnesses that lower our metabolic energy levels also lower our ability to synthesize the reducing equivalents that allow our body to bind and dispose of excess mercury. Mercury is known to inhibit the metabolic processes in mitochondria that produce ATP and NADH by inhibiting the enzymes of the citric acid cycle and the electron transport system. These nucleotides are absolutely required for both the synthesis of reduced glutathione and to reduce glutathione after it is oxidized. Glutathione in the reduced state is the major biomolecule involved in the natural removal of mercury from the body.

A recent publication supports our contention that mercury from dental amalgams poses a major threat to the exacerbation of AD. Olivieri et al. demonstrated that exposure of neuroblastoma cells to sub-lethal doses (36×10^{-9} molar) of Hg^{2+} caused both an increased secretion of b-amyloid protein and an increased phosphorylation of the microtubulin protein Tau (17). Both of these biochemical changes are uniquely observed in AD brain tissues and are considered diagnostic markers of the disease. b-amyloid protein makes up the 'amyloid plaques' that was one of the first diagnostic markers reported for AD brain pathology. A very strong component of AD researchers believe that amyloid protein is the cause of AD. Therefore, mercury exposure at nanomolar levels causes neuroblastoma cells to produce a protein that is believed to be involved directly in AD. This lead the authors of this paper to conclude that mercury would have to be consider as causal for AD (17). In a study published in the J. American Dental Association it was found that about 15% of the aged who died had mercury brain levels in the 10^{-6} to 10^{-7} molar levels. This is 100 to 1,000 times higher than what is required to cause the AD like aberrancies in neurons in culture as observed in the Olivieri study.

Further, the recent report of the response of neurons in culture rapidly forming neurofibrillary tangles on exposure to extremely low levels of mercury, by a process involving loss of microtubulin structure, completes the picture that mercury is capable of causing the formation of the two diagnostic hallmarks of AD in neuronal cultures (18). An impressive video accompanying this publication and available on the world wide web shows the addition of 2 microliters of 10^{-7} molar mercury to a 2 milliliter solution bathing neurons caused a rapid stripping of the tubulin from the neurofibrils leaving them bare.

The final concentration of mercury was 10⁻¹⁰ molar, or 10,000 times lower than levels found in 15% of aged brain samples. The bare neurofibrils could then wind and aggregate forming neurofibrillary tangles (NFTs) indistinguishable from those observed in AD brain and used as the major pathological diagnostic marker for AD. Just like our earlier studies, no other heavy metals other than mercury, could elicit this response. The final mercury concentration of 10⁻¹⁰ molar in these experiments is roughly 100 to 1000 times lower than the 10⁻⁷M levels found in most aged human brain of individuals with amalgam fillings even if they were not AD subjects. The majority of the mercury in brain is likely bound by protective proteins or selenium and not free to cause neuronal damage. However, these two recent publications supports the initial contention that mercury first rapidly inhibits enzymes like tubulin, creatine kinase and glutamine synthetase and dramatically affects metabolism and membrane structure. This leads rapidly to the formation of NFTs a diagnostic marker for AD. The exposure of the neurofibrils by the stripping of tubulin exposes the microtubular associated protein Tau to an aberrant situation leading to Tau's increased phosphorylation state as observed in AD brain tissue. These studies also suggest that a genetic susceptibility is involved.

After this occurs the cells responds to the cytotoxicity by producing and secreting amyloid protein, that forms the amyloid plaques observed on brain pathology and used to substantiate the AD diagnosis. To the point, neurofibrillary tangles, hyper-phosphorylated Tau, and amyloid plaques are the result of AD, not the cause. The cause is exposure to environmental toxicants like mercury that attack enzymes with the most reactive thiols groups. Wataha et al. (8) reported that extracts of the amalgam material (trade name, Dispersalloy) "was severely cytotoxic when Zn release was greatest, but less toxic between 48 and 72 hours as Zn release decreased". Zn is an essential metal needed for health and many times recommended by physicians to be taken in supplemental form. It is my opinion that the increased toxicity was not caused by direct Zn toxic effects. Rather, enhanced toxicity was due to the Zn potentiated toxicity of mercury caused by Zn²⁺ occupying biomolecule chelation sites resulting in a higher concentration of free Hg²⁺ capable of inhibiting the activity of critical nucleotide binding proteins such as tubulin and CK. This raises the question if mercury is released from amalgams under similar conditions. Chew et al. (9) tested the "long term dissolution of mercury from a non-mercury-releasing amalgam (trade name Compositel)". Their results demonstrated "that the overall mean release of mercury was 43.5 +/-3.2 micrograms/cm²/24hr, and the amount of mercury released remained fairly constant during the duration of the experiment (2 years)". In my opinion, this is not an insignificant amount of mercury exposure if one considers the number of years a 70 year old individual living today may have been exposed.

In our laboratory we soaked modern amalgam fillings in distilled water and then tested the resulting solution for toxicity. The results were obvious, the water was now extremely toxic and when added to brain homogenates dramatically inhibited the viability of tubulin and creatine kinase, exactly as observed when we added mercury cation. The bottom line is that mercury toxicity is enhanced by the presence of other heavy metals and both are released from dental amalgams. Additionally, when one considers the toxicity of a certain body level of mercury it is somewhat meaningless unless the body level of other heavy metals is also considered. In our work we also made amalgams outside of the mouth that were of known total weight and surface area. These were placed in sealed test tubes containing 50 ml of water. The water was changed daily and analyzed for mercury content. We calculated that about 4.5 micrograms mercury/cm²/day was released at room temperature. Brushing the amalgam for 30 seconds twice a day elevated

the mercury release about 10 fold.

Recent research has reported on damage to the olfactory nerve in the nasal cavity as a very-early indicator of AD (21-23). On its face, the olfactory nerve seems a likely avenue for Hg vapor from amalgams to directly access the brain, particularly due to the affinity of Hg to nerve tissue. It is sensible to expect mercury vapors from dental amalgams to adversely affect the olfactory nerves is one is to expect these same vapors to affect the brain neurons. The fact that reports exist indicating that loss of odor detection occurs before onset of AD symptoms is expected as the olfactory tissue is in direct contact with gases from the oral cavity.

Many recent literature and popular press reports state that the presence of periodontal disease raises the risk factor or exacerbates the condition of several other seemingly unrelated diseases such as stroke, low birth weight babies, cardiovascular disease (See October 1996 issue of Periodontology). The anaerobic bacteria of periodontal disease produce hydrogen sulfide (H₂S) and methyl thiol (CH₃SH) from cysteine and methionine, respectively. This accounts for the "bad breath" many individuals have. However, in a mouth that produces H₂S, CH₃SH (from periodontal disease) and Hg₀ (from amalgam fillings) the very likely production of their reaction products, HgS (mercury sulfide), CH₃S-Hg-Cl (methyl-thiol mercury chloride) and CH₃S-Hg-S-CH₃ (Dimethylthiol mercury) has to occur. This is simple, un-refutable chemistry whose presence is supported by easily observable amalgam tattoos. These tattoos are purple gum tissue surrounding certain teeth where the gum and tooth meet and caused by HgS as determined by mercury analysis of such tissue. HgS is one of the most stable forms of mercury compounds and is the mineral form of mercury, called cinnabar, from which mercury is mined from the earth). All of these compounds are classified as extremely toxic and the latter compound, dimethylthiol-mercury is very hydrophobic and its solubility similar to dimethyl-mercury. Dimethyl-mercury was the compound that was made well known in the press where only a small amount spilled on the latex gloves of a Dartmouth University chemistry professor caused severe medical problems and finally death 10 months later. Logic implies that anyone with periodontal disease, anaerobic bacterial-infected teeth and mercury-containing fillings would be exposed daily to these very toxic compounds. In our laboratory we synthesized the two methylthiol-mercury compounds (CH₃S-Hg-Cl and CH₃S-Hg-S-CH₃) and tested them. They are extremely cytotoxic at 1 micromolar or less levels and are potent, irreversible inhibitors of a number of important mammalian enzymes, including tubulin and CK.

To determine if toxic teeth could have an effect on the enzymes/proteins of human brain we have done the following study. Several very toxic teeth were incubated for 1 hour in distilled water. Aliquots of these solutions were then added to control human brain homogenates and the resulting samples tested for enzyme viability. The result showed that several of the solutions, but not all, in which toxic teeth had been incubated inhibited the viability of the same enzymes that are found to be inhibited in AD brain. Therefore, depending on the type of anaerobic bacterial infection in avital teeth it is possible to have a toxicant production that would exacerbate the condition classified as AD. Most likely, this toxicity would be more potent in the mouth of someone with co-existing dental amalgams.

In summary, the data on the effects of mercury on the nucleotide binding properties and the abnormal partitioning of two very important brain nucleotide binding proteins first suggested that mercury must be considered as a contributor to the condition classified as AD. This is strongly supported by the recent finds that nanomolar levels of mercury

causes neuroblastoma cells to secrete b-amyloid protein and increase phosphorylation of the microtubulin associated protein Tau, both major biochemical observations related to AD. Also, neurons in culture exposed to mercury cation at the 10^{-7} to 10^{-10} M levels have conclusively been shown to rapidly lose organized tubulin that surrounds the neurofibrils resulting in the formation of neurofibrillary tangles that are indistinguishable from those observed in AD brain and used as a diagnostic marker of the disease (18).

Consideration of mercury as an exacerbating factor is especially relevant when mercury is present in combination with other heavy metals such as zinc (Zn) cadmium (Cd) and lead (Pb). It has been reported that combining an LD-1 of lead with an LD-1 of mercury in rats gave an LD of 100 (19). Bluntly, the determination of safe body levels of mercury by using animal data where the animals have not been exposed to other heavy metals is no longer justifiable. Mercury is much more toxic to individuals with other heavy metal exposures. As I have been sent numerous lab reports on levels of elements in the hair and other tissues of suspected mercury toxic patients I have noticed that many have exceedingly high Pb, Cd, Cu, Zn, etc. levels. It is my opinion that one of the major questions left to be answered concerning the toxic effects of mercury is "does the combination of mercury with different heavy metals lead to different clinical observations of toxicity?" There can be no doubt that the elevated levels of other heavy metals increases the toxicity of mercury. Further, the reaction of oral mercury from amalgams and the reaction of this mercury with toxic thiols produced by periodontal disease bacteria very likely enhances the toxicity of the mercury being released. This makes any claim regarding the determination of safe levels of mercury as obtained under controlled conditions (e.g. in a system where other heavy metals are excluded) very suspect when discussing toxic mercury effects in the uncontrolled environment that humans are exposed to.

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