

Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury

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Christopher C. W. Leong; Naweed I. Syed; Fritz L. Lorscheider

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FROM ABSTRACT:

Inhalation of mercury vapor (Hg^0) inhibits binding of GTP to rat brain tubulin, thereby inhibiting tubulin polymerization into microtubules.

A similar molecular lesion has also been observed in 80% of brains from patients with Alzheimer disease (AD) compared to age-matched controls.

However the precise site and mode of action of Hg ions remain illusive.

Therefore, the present study examined whether Hg ions could affect membrane dynamics of neurite growth cone morphology and behavior.

Since tubulin is a highly conserved cytoskeletal protein in both vertebrates and invertebrates, we hypothesized that growth cones from animal species could be highly susceptible to Hg ions.

To test this possibility, the identified, large Pedal A (PeA) neurons from the central ring ganglia of the snail *Lymnaea stagnalis* were cultured for 48 h in 2 ml brain conditioned medium (CM). Following neurite outgrowth, metal chloride solution (2 l) of Hg, Al, Pb, Cd, or Mn (10^{-7} M) was pressure applied directly onto individual growth cones. Time-lapse images with inverted microscopy were acquired prior to, during, and after the metal ion exposure.

We demonstrate that Hg ions markedly disrupted membrane structure and linear growth rates of imaged neurites in 77% of all nerve growth cones.

When growth cones were stained with antibodies specific for both tubulin and actin, it was the tubulin/microtubule structure that disintegrated following Hg exposure. Moreover, some denuded neurites were also observed to form neurofibrillary aggregates.

In contrast, growth cone exposure to other metal ions did not effect growth cone morphology, nor was their motility rate compromised.

To determine the growth suppressive effects of Hg ions on neuronal sprouting, cells were cultured either in the presence or absence of Hg ions.

We found that in the presence of Hg ions, neuronal somata failed to sprout, whereas other metallic ions did not effect growth patterns of cultured PeA cells.

We conclude that this visual evidence and previous biochemical data strongly implicate Hg as a potential etiological factor in neurodegeneration. **[WOW!]**

THESE AUTHORS ALSO NOTE:

"Growth cones located at the tip of developing and regenerating neurites are responsible for neurite extension, axonal pathfinding and target cell selection in the nervous system."

"Actin and tubulin that comprise the bulk of growth cone cytoskeleton are highly sensitive to various environmental cues that are present in the extracellular milieu of growth cones."

"A growth permissive environment facilitates growth cone assembly whereas various growth inhibitory molecules disassemble microtubular structure, induce growth cone collapse and neurite retraction."

Microtubules are a principal protein of the cytoskeleton.

"Brain neurons require intact microtubules for axoplasmic transport, membrane structure, and normal neurite outgrowth."

"Methylmercury (MeHg) is a potent neurotoxicant, and its effects on microtubule integrity during CNS neuronal development are well documented."

"Attention has also focused on potential CNS toxicity resulting from chronic exposure to another predominant toxic mercury species, that of mercury vapor (Hg^0); the principal source being dental amalgam tooth fillings."

"Approximately 70% of all Hg ions in human urine originate solely from amalgam."

"Recently, we have reported that inhalation exposure of rats to Hg^0 causes disruption of brain microtubule metabolism by inhibiting the polymerization of tubulin molecules."

"Such polymerization is dependent upon the ability of GTP nucleotide to bind to tubulin, binding that is markedly reduced by the presence of Hg ions. A similar

in vivo molecular lesion was observed in brains of 80% of Alzheimer disease (AD) patients, but was not seen in brains from age-matched control patients.”

The amino acid sequence of tubulin from all animals brains (vertebrates and invertebrates) is highly conserved, with > 97% sequence homology across animal species, so using a snail neuronal culture model to study microtubule metabolism in the presence of Hg is acceptable.

The authors used a time-lapse imaging techniques for intact isolated neurons, using cell culture systems, which allowed the direct observation of axonal microtubule structure and protein synthesis at the neurite growth cone.

RESULTS

The authors tested for both immediate and chronic effects of Hg ions on neurite growth cone morphology and behavior.

All neurons cultured in the presence of brain conditioned media (CM) exhibited robust outgrowth over night.

“Within a few minutes of Hg exposure, not only did the growth cone cease its motility but it also exhibited robust collapse and retraction.”

The authors filmed their experiments: The film sequence showed dynamic neurite membrane disassembly and retraction following Hg exposure.

“The average linear growth rate for three of these growth cones was determined to be +28 m/h before Hg exposure, compared to -102 m/h during and -146 m/h after Hg exposure.” **[WOW!]**

The authors “have repeated this experiment with similar results for 40 different neuron cultures under the same conditions over a 2-year period. In these cultures, on average, 77% of all nerve growth cones were affected by Hg.”

The authors tested other metallic ions (Al, Pb, Cd or Mn), and despite multiple exposure to the above ions, “the growth cone morphology and behavior remained unperturbed suggesting that these ions do not affect growth cone cytoskeleton.”

“The Hg ion treated growth cones exhibited a high degree of disintegration of tubulin/microtubule structure.”

These findings demonstrate that Hg ions exert growth suppressive effects on the growth cone of neurons.

"Neurons cultured in the presence of Hg ions failed to initiate neurons ($4.6 \pm 2.4\%$ sprouting), whereas control neurons extended robust outgrowth ($93.4 \pm 3.1\%$ sprouting).

The "effects of Hg ions are not restricted to individual growth cones, rather they prevent neurite initiation from the entire neuron."

DISCUSSION

"The results of the investigation described herein clearly demonstrate that exposure to Hg ions markedly disrupts the membrane structural integrity of neurites and the growth cones of identified neurons."

"This phenomenon appears to be specific for Hg, since exposure to four other heavy metals had no observable effect on either growth cone morphology or individual neurites."

A number of neurobehavioral effects in dental personnel resulting from chronic low-level exposure to Hg⁰ has been reported.

These authors suggest that Hg disrupts the integrity of the neurite membrane in growth cones of intact neurons, which may implicate Hg as a potential etiological factor in neurodegeneration that could ultimately be observed as altered neurobehavior.

Mercury induced growth cone collapse: another reason for flossing

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Owen Hamill

Occupational poisoning in workers of the 19th century hat industry due to mercury as a stiffener of rabbit fur, resulted in irritability, mood swings, tremulousness, ataxia and impairment in intellectual capacity, termed Mad Hatter's disease.

"Currently there is ongoing public health debate on whether low level chronic exposure to mercury due to dental repair work results in subclinical behavioral changes associated with CNS damage."

"In the USA the most common material used in dental fillings is a mercury/silver mixture (amalgam) in which an estimated 70,000 kg is used in 100 million fillings/year."

"Evidence indicates that mercury vapor is continuously released from tooth fillings where it is breathed in by the lungs and converted into mercuric ions."

What are the more subtle, pre-clinical effects associated with chronic low level mercury exposure in the general population with fillings?

This study (Leong) shows that "exposure to mercury concentrations of < 0.1 M results in rapid (i.e. within 10 min) retraction of growth cones in snail neurons and is correlated with disruption of microtubules."

"The authors point out that similar disruption of microtubules is associated with Alzheimer's disease."

"These recent findings give added impetus for the development and implementation of alternative materials for fillings and may provide parents with added ammunition in teaching their children to floss."

KEY POINTS FROM DAN MURPHY

- (1) This is the best evidence I have seen to date relating the potential for mercury/silver mixture (amalgam) dental fillings to cause neurodegeneration including Alzheimer's Disease.
- (2) Low level mercury exposure not only stopped the development of new synapses, it destroyed existing synapses.
- (3) Apparently, the exposure from the Hg is through the vapors that emanate throughout the rest of one's life.

COMMENTS FROM DAN MURPHY

You can actually view the neurite destruction at the web site for these authors: <http://movies.commonscalgary.ca/mercury>

When I showed this article to my dentist, he had already seen it, and stated: "I will never again insert a mercury/silver mixture (amalgam) filling."

This article was originally given to me by Gerald Clum, DC, President of Life Chiropractic College West. I first read about it in Dr. Clum's free email newsletter. If you would like to receive Dr. Clum's free email newsletter, email gclum@lifewest.edu and ask to be added to TLS in the heading.