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Mercury in human spinal motor neurons

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Abstract Inorganic mercury has been proposed as a neurotoxin that could cause sporadic motor neuron disease (SMND). We were therefore interested to see if mercury could be detected in the upper and lower motor neurons of SMND patients, and if mercury accumulated within motor neurons during life. Paraffin sections of formalin-fixed spinal cord (22 control adults, 20 SMND adults, 25 infants) and frontal primary motor cortex (9 control adults, 18 SMND adults, 20 infants) were stained with silver nitrate autometallography to detect ionic mercury. Mercury was found in the spinal motor neurons of 36% of adult control cases and 45% of adult SMND cases, with no significant difference between groups. No mercury was seen in infant spinal motor neurons, or in any adult or infant corticomotoneurons. In conclusion, many humans appear to accumulate mercury in their spinal motor neurons by the time they are adults, but mercury does not appear to play a major role in the loss of upper or lower motor neurons in SMND.

Key words Amyotrophic lateral sclerosis · Mercury · Motor neuron disease · Neurotoxicity

Introduction

It has been suspected for some time that heavy metals play a role in the pathogenesis of sporadic motor neuron disease (SMND), also known as amyotrophic lateral sclerosis [16]. Unfortunately, the difficulties of demonstrating small amounts of metals within neurons has led to conflicting reports of concentrations of lead and mercury in nervous tissues of SMND patients [6]. Largely because of this, support for a heavy metal hypothesis for SMND has waned in the last few years.

New impetus for the idea that inorganic mercury could cause motor neuron loss has come from studies using the technique of autometallography which detects small amounts of ionic mercury within cells [7]. When experimental animals are exposed to high or low doses of inorganic mercury (given either as mercuric chloride or as mercury vapour) the mercury demonstrated by autometallographically localizes particularly but not exclusively to motor neurons in the spinal cord and brain stem [17–20]. When experimental animals are exposed to methylmercuric chloride, on the other hand, mercury is found in many neurons throughout the nervous system [17], probably because methylmercuric compounds readily cross the blood-brain barrier [5]. Mercury has been claimed to have been seen in spinal motor neurons in normal humans using an autometallographic technique, but this finding has only been mentioned in passing in a review article [13]. No thorough study of the ionic mercury content of normal or diseased motor neurons in humans appears to have been undertaken.

In determining whether a neurotoxin could be responsible for SMND it is important to see if the toxin can be found in both cortical and spinal motor neurons, since both are involved in SMND [3]. Most animal experiments of inorganic mercury uptake have been performed in rodents, which unlike primates do not have corticomotoneurons directly synapsing with spinal motor neurons [23], so a rodent model is unsuitable to test for cortical motor neuron pathology. Primate models are therefore more relevant when considering cortical motor neurons. When a monkey was implanted with dental amalgam fillings containing radioactive mercury, some mercury could be detected in the frontal cortex and other brain regions [11]. When monkeys were exposed to mercury vapour, mercury was detectible in the brain using an autometallographic technique, with the greatest amount located in large motor neurons in the precentral gyrus [28]. In one man exposed to a large dose of metallic mercury, mercury was found selectively in frontal corticomotoneurons [21], but it is

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not known if low doses of inorganic mercury enter human corticomotoneurons. The evidence therefore suggests that primate cortical motor neurons, as well as spinal motor neurons, may take up inorganic mercury selectively.

With mercury being implicated in motor neuron pathology, we looked for mercury in spinal and cortical motor neurons of SMND and control cases. In addition, tissue from infants was examined to see if mercury in motor neurons was present at birth or whether it accumulated during postnatal life.

Materials and methods

Experimental groups

Tissue stored in the Department of Pathology brain bank was examined from the following cases.

1. Adult non-SMND cases. In this group were 22 subjects who died from conditions other than SMND (15 male, 7 female, age range 26–86 years, mean age 65.0 years). Causes of death were Alzheimer's disease (5), cancer (4), ischemic heart disease (2), and single cases of cystic fibrosis, infective endocarditis, pneumonia, olivopontocerebellar degeneration, Wilson's disease, paraneoplastic encephalomyelopathy, multiple sclerosis, trauma, meningitis, multisystem atrophy, and cor pulmonale. No subject was known to have worked in an industry using mercury, and no dental records were available. The spinal cord was available in all cases and the frontal motor strip in 9 cases.

2. Adult SMND cases. This group consisted of 20 subjects who died of SMND (12 male, 8 female, age range 38–76 years, mean age 64.3 years). All had been seen in life by a neurologist who made the diagnosis of SMND on the basis of progressive weakness with both upper and lower motor neuron signs and no involvement of other neurological systems. No subject was known to have worked in an industry using mercury and no dental records were available. The diagnosis of SMND was confirmed pathologically by finding spinal motor neuron loss and variable corticospinal tract degeneration. The spinal cord was available in all cases and the frontal motor strip in 18 cases.

3. Infant cases. In this group were 25 infants (17 male, 8 female, postnatal age range 3–75 weeks, mean postnatal age 21.2 weeks) who died from either the sudden infant death syndrome (17 infants) or from known medical causes (8 infants). The spinal cord was available in all cases and the frontal motor strip in 20 cases.

Tissue processing and staining

The brain and spinal cord were removed at postmortem examination and immersion-fixed in 15% neutral-buffered formalin for 2 weeks. A transverse block of cervical spinal cord (centred on C7) and a horizontal block containing the frontal primary motor cortex (3 cm from the vertex) were removed as previously described [22]. Blocks were processed routinely for paraffin embedding and from each block two 7-μm sections, separated by 14 μm, were cut. Two sections were chosen because these gave between about 60–100 motor neurons for examination in control cases, and the same number of sections were used in SMND cases for comparative purposes. Both sections were stained with autometallography to detect ionic mercury. Briefly, sections were placed in physical developer comprising 60 ml of 50% gum arabic, 10 ml citrate buffer, 30 ml of 5.6 g/100 ml hydroquinone and 0.5 ml of 17 g/100 ml of silver nitrate, and kept in the dark at 26° C for 80 or 90 min [9]. Sections were counterstained with 0.5% cresyl violet. Parallel sections of silver-staining sections were pretreated for 2 h with 1% potassium cyanide to eliminate silver contamination of tissue [8]. Sections were viewed with an Olympus BX50 microscope using transmitted bright-field and dark-field illumination, as well as reflected ultraviolet light fluorescence. Silver-enhanced deposits stable after pretreatment with potassium cyanide are referred to as "mercury granules".

In each spinal cord section the number of motor neurons (defined as cell bodies in the anterior horn ≥ 25 μm in greatest diameter with prominent Nissl substance) containing mercury was divided by motor neurons not containing mercury to give a proportion of mercury-containing motor neurons.

Results

Adult non-SMND cases

Among the 22 non-SMND subjects, mercury was seen in 8 cases (36%) in 5–20% of large spinal motor neurons. The mercury granules were present in the cell bodies of scattered motor neurons in the lateral anterior horns of the cord, with closely adjacent motor neurons usually showing no mercury (Figs. 1, 2). In no case did the silver-enhanced granules disappear on pretreatment with potassium cyanide. Within the neurons, mercury granules appeared to be clustered within lipofuscin; however, most lipofuscin-laden neurons did not contain mercury granules (Figs. 2, 3). Of 11 cases with neurological disease, 5 had spinal motor neuron mercury versus 3 of 11 cases without neurological disease (no significant difference on Fisher's exact 2×2 contingency test).

No mercury was found in any corticomotoneurons in the 9 frontal motor strips available (Figs. 4, 5) despite most of these neurons containing large amounts of lipofuscin (Fig. 6). Four of these cases had spinal motor neurons containing mercury.

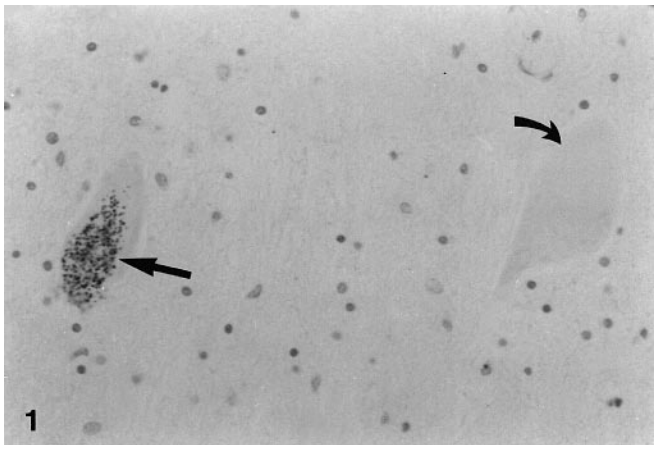

Fig. 1 Bright-field illumination of two spinal motor neuron cell bodies *(arrows)* from a 74-year-old man who died with Alzheimer's disease. Black mercury granules are clustered in the cell body of one neuron *(straight arrow)*. No mercury granules are seen in an adjacent motor neuron *(curved arrow)*. Autometallography (with potassium cyanide pretreatment) and cresyl violet. × 330

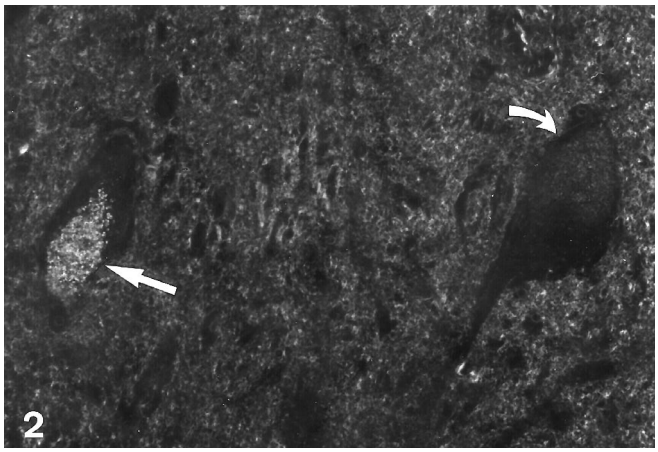

Fig. 2 Same spinal neurons as in Fig. 1. On dark-field microscopy, reflective mercury granules stand out prominently *(straight arrow)*, whereas lipofuscin alone reflects light poorly *(curved arrow)*. Autometallography (with potassium cyanide pretreatment) and cresyl violet. × 330

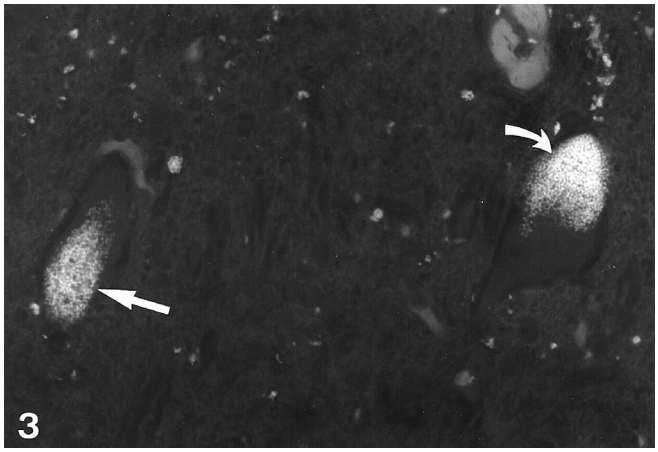

Fig. 3 Same spinal neurons as in Fig. 1. Lipofuschin shows ultraviolet light autofluorescence in neurons both with mercury granules *(straight arrow)* and without mercury granules *(curved arrow)*. Autometallography (with potassium cyanide pretreatment) and cresyl violet. × 330

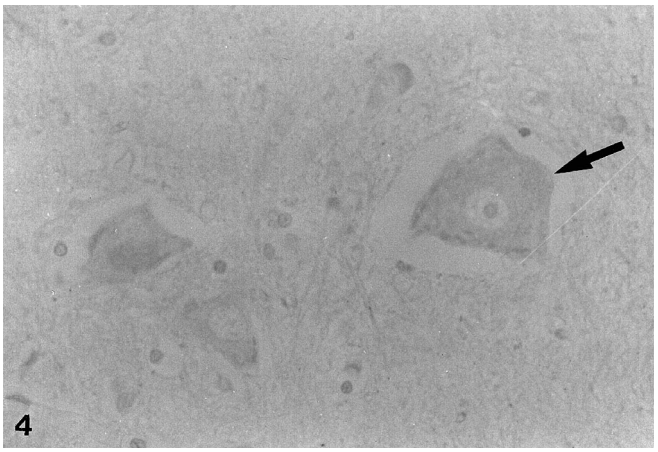

Fig. 4 Cell body of a large cortical motor neuron *(arrow)* from the same case as in Fig. 1. No dark mercury granules are seen on bright-field illumination in this or in the two smaller neurons on the left. Autometallography and cresyl violet. × 395

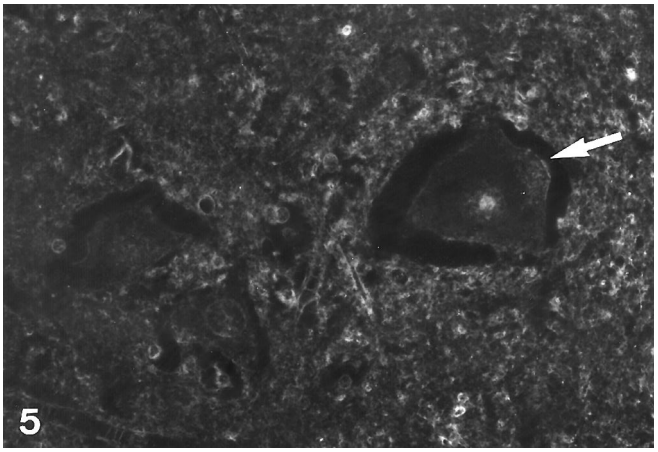

Fig. 5 Same cortical neurons as in Fig. 4. No brightly reflective particles are seen on dark-field microscopy, though lipofuscin reflects some light in one part of the large neuron *(arrow)*. Autometallography and cresyl violet. × 395

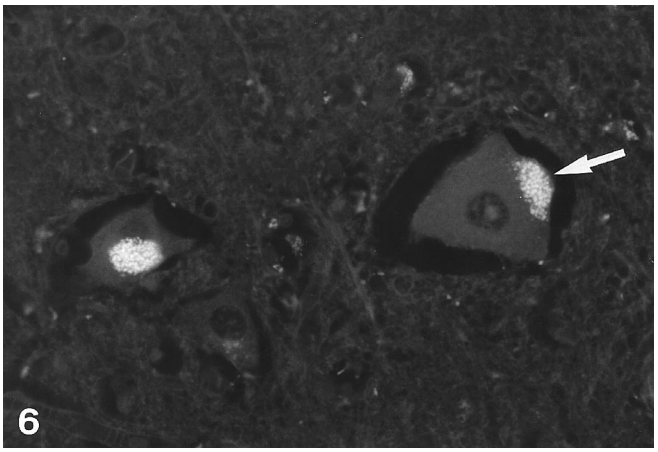

Fig. 6 Same cortical neurons as in Fig. 4. Lipofuschin shows bright ultraviolet light autofluorescence in the large *(arrow)* and smaller neurons. Autometallography and cresyl violet. × 395

Adult SMND cases

In the 20 SMND subjects, mercury granules were seen in 10–30% of the remaining spinal motor neurons in 9 cases (45%). Mercury was not found in any remaining corticomotoneurons in the 18 frontal motor strips examined, although 9 of these cases had spinal motor neurons containing mercury.

Differences between groups

The numbers of individuals with mercury-containing spinal motor neurons did not differ significantly at the 5% level between SMND and non-SMND cases (Fisher's exact 2 × 2 contingency $P = 0.33$). The presence of mercury in spinal motor neurons did not relate significantly to age

(cases with mercury mean age 64.1 years, without mercury mean age 64.2 years, Student's $t = 0.02$, $P = 0.98$), postmortem delay (cases with mercury mean delay 30.7 h, without mercury mean delay 33.4 h, Student's $t = 0.32$, $P = 0.74$), or gender (Fisher's exact 2×2 contingency $P = 0.75$).

Infant cases

No mercury granules were found in any of the spinal motor neurons (25 infants) or corticomotoneurons (20 infants) examined.

Discussion

Inorganic mercury is an attractive candidate for a cause of SMND since it enters motor neurons selectively at low doses [20], probably because it can bypass the blood-brain barrier by entering terminal motor axons via neuromuscular junctions [1]. Ionic mercury could damage neurons by generating oxygen radicals in mitochondria [14] or by decreasing the astrocytic uptake of glutamate [2], both mechanisms that have been implicated in motor neuron disease [29]. People who have been exposed occupationally to mercury and other heavy metals appear to be at increased risk of getting SMND [24] and SMND sometimes follows accidental exposure to inorganic mercury [26]. Humans are exposed to mercury ions in water and food, and to mercury vapour (which is converted to mercury ions in red blood cells [15]) from industrial pollution and dental amalgam [5]. It has, however, been difficult to determine whether motor neurons in SMND contain mercury using standard methods of mercury analysis since these either destroy the tissue or are insufficiently sensitive. The technique of autometallography on the other hand has enabled workers to localize ionic mercury within neurons of humans exposed to inorganic mercury [12, 21]. Using this technique we have shown that scattered spinal motor neurons in many adults contain ionic mercury.

The results of this study overall do not favor the hypothesis of mercury-induced neuronal loss in SMND. The finding in particular on semiquantitative analysis that the proportion of mercury-containing motor neurons did not differ between control and SMND groups argues against ionic mercury by itself being a major pathogen in SMND. This implies that for mercury to cause neuronal loss, SMND patients must have some added susceptibility to the toxic effects of intraneuronal mercury. It is possible that we underestimated the number of subjects with neurons containing mercury because we examined only two spinal cord sections, but if mercury were to be found in only a few neurons after many sections were examined it would probably be biologically insignificant. Secondly, fewer than half of the SMND cases had motor neurons containing mercury. It could be postulated that in these SMND cases the remaining neurons are less likely to contain a neurotoxin such as mercury (a "survivor" effect), but this is only speculative. Thirdly, mercury was not found in any corticomotoneurons, suggesting that the mercury in spinal motor neurons was not passed on to upper motor neurons transynaptically. This argues against ionic mercury causing SMND since quantifiable corticomotoneuron loss is a feature of this condition [22]. It is, however, theoretically possible that in SMND either the spinal or the cortical motor neuron could die first, followed by transynaptic degeneration of the connecting neuron [10]. Therefore, if mercury injured spinal motor neurons the directly-synapsing corticomotoneurons could undergo retrograde loss. The significance of each of our three findings could, therefore, be interpreted in contrary ways, but taken together they do not support a role for mercury in most cases of SMND.

The failure to find mercury in motor neurons of infants indicates that mercury does not commonly enter human fetal motor neuron from placental transfer. This is despite the fact that mercury vapour has been shown to cross the placenta readily in experimental animals [4] and that blood mercury levels in the sheep fetus may be fourfold higher than in the mother implanted with dental amalgam [27]. The mercury found in the spinal motor neurons of the human adults in this study must, therefore, have accumulated during postnatal life. In experimental animals repeated low doses of ionic mercury have been shown to accumulate until mercury eventually becomes visible in motor neurons [25]. A similar accumulation of ionic mercury from industrial pollution or dental amalgam exposure, with retrograde transport of the mercury ions from the motor axon terminal to the cell body, could explain the finding of mercury in the spinal motor neurons of the adults in this study. Unfortunately, we were not able to determine the life-long exposure of these subjects to dental amalgam because detailed long-term dental records were not available. Many subjects had no or few remaining teeth so the number of amalgam fillings at the time of death would not give an accurate estimation of mercury exposure from fillings during life.

In conclusion, it appears that many humans accumulate enough ionic mercury in their spinal motor neurons to become visible histochemically in adulthood. From this study, damage to motor neurons from cytoplasmic mercury does not appear likely, although motor neuron damage in people with a particular susceptibility to intraneuronal mercury cannot be ruled out. Given the known neurotoxicity of mercury, however, it seems prudent to continue attempts to reduce human exposure to ionic mercury to the lowest possible levels.

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