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Role for apolipoprotein E in neurodegeneration and mercury intoxication

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1. ABSTRACT

Mercury intoxication is a serious public health problem and a worldwide concern. The Minamata Convention on Mercury has been signed by 128 countries and endorsed by the World Health Organization with the recommendation of promoting the management of epidemiological information. The Central Nervous System is the main target organ for mercury. Symptoms of intoxication include altered motor coordination, visual and tactile dysfunction and paralysis, caused by neurodegeneration with a key role for oxidative damage. Recently, some studies have demonstrated a correlation between mercury intoxication and isoforms of apolipoprotein E (ApoE). In this review, epidemiological data and hypotheses about the possible molecular mechanisms underlying the association between ApoE and mercury intoxication are assessed. Based on the evidence and the neuropathological changes that the presence of ApoE4 and mercury neurotoxicity have in common, we propose a convergent action of both factors. ApoE4 seems to potentiate the damage caused by mercury. Increased knowledge of this interaction using epidemiological and pre-clinical studies is essential to improve prevention strategies to adequately manage intoxicated patients.

2. INTRODUCTION

The etiology of many neurodegenerative disorders is still unclear and the role of the environment as a putative risk factor is being increasingly studied. Exposure to heavy metals, for example, is now recognized as a potential etiologic factor for some of them, such as Alzheimer's disease (AD) (1, 2); Parkinson disease (3) and amyotrophic lateral sclerosis (4). Mercury, aluminum, cadmium and arsenic have been studied in AD due to their ability to increase amyloid-beta (Aβ) peptide and to produce

abnormal forms of tau protein, causing senile/amyloid plaques and neurofibrillary tangles, respectively (1). Potential gene-environment interactions have been investigated extensively in the pathogenesis of some of these diseases. For example, apolipoprotein E4 (ApoE4) is the only genetic risk factor confirmed to play a role in the development of late onset Alzheimer's disease, increasing the risk level by three-fold in heterozygous individuals and twelve-fold in homozygous individuals (1). However, the possible role of apolipoprotein E (ApoE for the protein; *APOE* for the gene) in other neurodegenerative conditions is not well understood.

Although ApoE has been associated to the effects of metals such as mercury, lead, zinc, copper and iron, this relationship seems to be mainly toxicokinetic, (i.e. ApoE isoforms influencing the bioavailability and the clearance of these metals) (1,5-7), except for mercury. Among these metals, mercury and lead have studies in humans about the association between ApoE isoforms and the deleterious consequences of metal intoxication (5), but only mercury show many works demonstrating a well-established epidemiological correlation.

Neurodegeneration due to mercury intoxication is an important concern, especially in the Amazon region (8-10). Moreover, mercury is a ubiquitous metal responsible for many episodes of environmental contamination in Brazil and throughout the world. In the last decade, some authors have indicated a possible role for the different ApoE isoforms in individual susceptibility to mercury intoxication (11-16). Therefore, this review aimed to analyze the influence of ApoE in the pathogenesis of mercury neurodegeneration and to describe the hypotheses for the possible molecular mechanisms underlying the relation between ApoE and mercury neurotoxicity.

3. MERCURY: A WORLDWIDE CONCERN

In October 2013, diplomatic participants from 91 countries met in Kunamoto (Japan) and signed the Minamata Convention on Mercury (www.mercuryconvention.org), an international treaty to join efforts with the aim of reducing and combating environmental and human exposure to mercury. Presently, 128 countries have signed the Convention and 29 countries have ratified it. The World Health Organization (WHO) has recognized and supported this action, publishing a resolution to endorse the Convention (17). In this resolution, the WHO recommends analyzing aspects related to human health in order to promote adequate health care for prevention and the treatment of affected populations and to facilitate the collection and exchange of epidemiological information about the impact of mercury exposure health.

Mercury can be found in the environment from both natural and artificial sources. Human activities such as the burning of fossil fuels, chlor-alkali industries or traditional gold mining can result in episodes of acute human intoxication. These anthropogenic activities provoke contamination of the environment with the potential of also affecting populations living far away from the origin of pollution (10). Additionally, mercury can be naturally found in soils (usually as cinnabar) and volcanic emissions (18). Other anthropogenic activities, beyond those directly related to industry include river damming or deforestation, and have the potential of mobilizing and accumulating natural mercury (18). Biotransformation by methanogenic archaea and biomagnification through the food chain are the major processes responsible for the availability of methylmercury for human exposure (10). Methylmercury, one of the most toxic species of mercury, easily cross lipid membranes to reach its main target organ, the central nervous system (CNS) (9). Major symptoms of human intoxication include altered motor coordination, visual and tactile dysfunction, and paralysis. The neurodegeneration responsible for these symptoms is mediated by oxidative damage (9, 19). Oxidative damage to macromolecules (proteins, lipids and DNA) has been demonstrated in mercury intoxication with deleterious consequences, especially for the brain, because of the high metabolic activity of this organ and the relatively low content of antioxidant defenses (8).

Mercury toxicity varies according to the route of entry, the amount of exposure and individual susceptibility (20). The main route of mercury intake in occupational exposure is via inhalation, but episodes of human intoxication caused by chronic consumption of contaminated food (especially piscivorous fish at the top of food chain (21)) are the main type of exposure for methylmercury (10). Thus, the WHO has established a safety limit for mercury content in human hair (the main sample used for the

evaluation of methylmercury exposure) of 10 µg/g or below (22). Still, this limit is based on acute outbreaks such as those in Minamata and Iraq; some studies have shown in recent years that exposure to relatively low levels of methylmercury (below the WHO limit) have the potential to cause additional long-term consequences such as genotoxicity (9, 23-25). Although recent studies on AD patients or older individuals have failed to find correlations between the content of mercury and neurodegenerative processes (2, 26), chronic neurodegeneration due to chronic methylmercury exposure may be better associated with longitudinal assessments of mercury levels (7,11-16).

Additional to the continuous monitoring of contaminated environments and human populations, some gene-environment interactions were already described in epidemiological studies affecting the toxicokinetics or toxicodynamics of methylmercury (see Table 1). Efforts have focused on finding genetic biomarkers of susceptibility to mercury intoxication in order to develop prevention strategies and to identify high-risk individuals for early intervention. In the last decade, an important role for apolipoprotein E as a genetic susceptibility factor against the mercury intoxication has been found (11-16). Thus, in humans, a strong association between the different isoforms of ApoE and individual susceptibility to mercury intoxication exists, such that individuals containing one or two copies of the *APOE epsilon 4* allele being more susceptible to damage (11, 13).

4. NEUROBIOLOGY OF APOLIPOPROTEIN E

Apolipoproteins are the protein component of lipoproteins, such as chylomicrons (Qm), very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and high density lipoprotein (HDL). Apolipoproteins solubilize lipids and facilitate the transport of these hydrophobic molecules in an aqueous medium, like plasma (27). As a part of these molecules, apolipoproteins are peripherally located in the molecule surface (such as in the case of apolipoproteins A, C and E) or they are trasmembrane reaching the lipid core of the lipoprotein (as in the case of apolipoprotein B) (27, 28).

Apolipoprotein E is a glycoprotein of 34 kDa containing 299 amino acids (29-31). ApoE is a key apolipoprotein that regulates lipid metabolism in the whole body and, in the brain, it is able to modulate the delivery of cholesterol to neurons. ApoE circulates in the blood as a component of VLDL, chylomicrons and a subclass of HDL. In the cerebrospinal fluid (CSF) and CNS, ApoE circulates as small particles or disks that resemble the peripheral HDL component (32).

The brain has the highest content of cholesterol in the entire human body (approximately 25% of all cholesterol) and ApoE is the major apolipoprotein found in this tissue (33). The brain is also the second largest site of synthesis of ApoE, mainly produced by astrocytes to transport cholesterol to neurons via ApoE receptors. Moreover, its synthesis is essential for axonal growth, synaptic formation and remodeling. All cholesterol present in the CNS is synthesized *in situ* by *de novo* synthesis. Cholesterol from the periphery virtually does not cross the blood-brain barrier, making an adequate synthesis and homeostasis essential in the CNS (34). Further, ApoE can also be synthesized by neurons, usually in response to insult or injury, to promote integrity and neuronal repair (32).

In humans, ApoE is encoded by a polymorphic gene, located on the long arm of chromosome 19 at position 13.2., with 3.7. kilobases, four exons and three introns (35, 36, 29). There are three major isoforms of ApoE (ApoE2, ApoE3 and ApoE4), encoded by the alleles *epsilon 2*, *epsilon 3* and *epsilon 4*, respectively (35, 36, 29), with six possible combinations of genotype (*epsilon 2/epsilon 2*, *epsilon 2/epsilon 3*, *epsilon 2/epsilon 4*, *epsilon 3/epsilon 3*, *epsilon 3/epsilon 4*, *epsilon 4/epsilon 4*). Although sequencing analyses have demonstrated that the *epsilon 4* allele is the ancestral one, the *epsilon 3* allele increased its frequency during the evolutionary process and it is presently the most common isoform. The mean frequencies found in the general population are 13.9.%, 79% and 7.3.% for ApoE2, E3 and E4, respectively (37).

These three isoforms differ in the content of two amino acids at residues 112 and 158 of the protein (11, 29). ApoE3 has one cysteine (Cys) and one arginine (Arg) at positions 112 and 158, respectively. ApoE2 has cysteine residues at these two positions and ApoE4 has arginine residues (Figure 1).

Despite these minor changes in the primary chain, they have important effects on both the secondary structure and the function of ApoE. These effects modulate the association between the different genotypes of ApoE and longevity or some diseases, such as Alzheimer's disease (35, 36, 31, 32). The *epsilon 4* allele of the *APOE* is the strongest risk factor for both early-onset and late-onset AD; its frequency is dramatically increased to nearly 40% in these patients (38). ApoE2 is associated with a reduced probability of developing AD and a delay in the onset of AD (30). There are several hypotheses about the association between the presence of ApoE4 and the neurodegeneration found in AD: ApoE4 may increase tau hyperphosphorylation, facilitating the formation of neurofibrillary tangles (39); also, it may favor the deposition and production of Aβ and impair the clearance of Aβ, leading to the accumulation of senile plaques (39, 40). An important pathway of Aβ clearance is via the low-density lipoprotein receptor-related protein-1 (LRP1), located at the blood-brain barrier (BBB) (41, 42). This is an ApoE isoform-dependent mechanism with ApoE4 showing a major deleterious effect on Aβ clearance (43). The complex Aβ-ApoE4 is eliminated via VLDLR in a slower way than the clearance of Aβ-ApoE2 and Aβ-ApoE3 complexes by LRP1(43).

Several studies have shown differences in the roles of ApoE3 and ApoE4 in neurodegeneration, showing protective and deleterious effects for each isoform, respectively. In ApoE3 individuals, clearance of Aβ, protection against tau protein phosphorylation, cholesterol efflux, stimulation of neurite growth and neuroprotection against cognitive decline have been demonstrated (40). In contrast, ApoE4 subjects show increased deposition of Aβ, neurite growth inhibition, breaks in the neuronal cytoskeleton, stimulation of tau protein phosphorylation, neurodegeneration and cognitive decline (31, 40). ApoE deficiency is associated with increased neurodegeneration during aging. Also, the dramatically increased synthesis of ApoE produced both in the CNS and in the peripheral nervous system (PNS) is observed after damage (44). Thus, ApoE seems definitely help in the repair and/or protection of neurons through mechanisms that remain unknown (44).

Interestingly, *epsilon 4* carriers display the lowest levels of both blood and brain ApoE, when compare to those of *epsilon 2* and *epsilon 3* carriers, and a tendency toward reduced longevity in these individuals (35, 36, 47). This fact has been associated with the antioxidant properties of ApoE that were demonstrated by *in vivo* and *in vitro* studies (47). In ApoE4 individuals, this protection may be reduced because of decreased levels of ApoE, in addition to a limited capacity to adequately respond to oxidative processes (47). These characteristics may lead to the exacerbation of oxidative damage, especially in the brain, thus facilitating aging processes and decreasing the longevity of ApoE4 carriers (47). Oxidative damage is also a main molecular mechanism present in some neurodegenerative conditions such as mercury intoxication (8).

5. APOLIPOPROTEIN E4 AND MERCURY POISONING

ApoE is the only apolipoprotein that has been associated to the deleterious consequences of mercury exposure. No other apolipoprotein gene has been associated to the susceptibility to mercury intoxication. Interestingly, the association between ApoE isoforms and the extension of the injury caused by mercury poisoning in humans has been already demonstrated in epidemiological studies (7, 11, 13-16). However, presently, there are only theories to explain a possible cause and effect.

The first mechanism proposed by Pendergrass and Haley in 1995 (45) suggests that ApoE4 individuals (genotypes: *epsilon 3*/*epsilon 4* and *epsilon 4*/*epsilon 4*) intoxicated with mercury would have ApoE with decreased ability to bind or chelate the metal compared to individuals presenting the ApoE2 or ApoE3 isoforms. This phenomenon may facilitate the presence of the free form of the metal, allowing it to remain available and exert its toxic effects (45). This biochemical explanation was proposed based on the differences in the amino acid composition of the three isoforms of ApoE and the affinity of mercury for the sulfhydryl groups of proteins. Cysteine residues of the ApoE molecule bear sulfhydryl groups that are

absent in arginine residues. So, ApoE2 individuals (ApoE has two cysteines at positions 112 and 158) may have a greater capacity to remove free mercury from the CSF and the brain than ApoE3 (ApoE with only one cysteine at 112) and ApoE4 (with arginines at the two positions) individuals. According to this idea, ApoE4 would show the lowest capacity to bind and remove mercury, leading to metal accumulation in these tissues. (Hybrid 50% capacity to remove)

After that, several authors demonstrated a higher frequency of the *epsilon 4* allele in groups formed by individuals with higher levels of mercury and/or symptoms of mercury intoxication (7, 11, 13-16, 45).

Godfrey and colleagues (2003) studied the relationship between ApoE and mercury intoxication, and they established ApoE genotype as a possible biomarker for mercury neurotoxicity. The study was conducted with 400 patients showing neuro-psychiatric symptoms associated with mercury intoxication that were compared with control individuals without symptoms. They found a significantly higher proportion of symptomatic patients exhibiting the *epsilon 4/epsilon 4* in genotype comparison with the control group. The *epsilon 2/epsilon 2* and *epsilon 2/epsilon 3* genotypes were less frequent in the symptomatic group (11).

Another strong correlation between ApoE4 individuals and the characteristic symptoms of chronic mercury toxicity, Alzheimer's disease, bipolar disorder and depression was found in populations exposed to mercury from the amalgam of dental restorations (13). Thus, the presence of ApoE4 may have the potential to increase the risk of mercury intoxication, whereas ApoE2 individuals may show the lowest risk (13).

The high entropy of mercury for the human brain and its ability to cross any lipid membrane (including placenta barrier) mean that special attention should be paid to the effects of the metal in the neurodevelopment of fetus and children. An interesting study with a cohort of Taiwanese children that were assessed from birth to two years old demonstrated that prenatal mercury exposure was associated with significant adverse effects on cognition, social behavior and neurodevelopment among carriers of at least one copy of the *epsilon 4* allele (14). Following this cohort, these authors posteriorly detected that an increased mercury content in cord blood enhanced the risk of deficit behavior in preschool children who were *epsilon 4* carriers (15). Recently, another cohort with 300 children of Lisbon, Portugal, aged 8 to 12 years and exposed to mercury from amalgam, found that mercury exposure may especially affect neurobehavioral function (learning, memory, attention and motor coordination) in ApoE4 children (16). Therefore, the results of the latter study (Table 2) and the affinity of mercury for the sulfhydryl groups present in the cysteine residues of ApoE may support the correlation between the inability of ApoE4 individuals to eliminate mercury (*epsilon 3/epsilon 4* and *epsilon 4/epsilon 4*) and the increase in the incidence and intensity of the symptoms of mercury toxicity. However, other possibilities must not be discarded.

The small differences in the amino acid composition of ApoE isoforms permanently influences the spatial conformation of the ApoE molecule. It has been proposed that, in ApoE4, the arginine at position 61 interacts with glutamate at position 255, meaning that the N-terminal domain approaches the C-terminal domain due to the reorientation provoked in the molecule (Figure 2) (46, 48). This may not happen with the ApoE2 and ApoE3 domains where position 112 is occupied by cysteine residues which interact with the arginine at position 61, preventing the interaction with glutamate 255 (46).

In addition to this important interaction between the helices, recent nuclear magnetic resonance, fluorescence and computational simulation data indicate several interactions between the N- and C-terminal domains with a higher stabilizing effect in ApoE3 when compared to ApoE4 (49-52). Moreover, the different isoforms may be characterized by different misfolded intermediate states, thereby influencing lipoprotein binding sites and resulting in different functional activity and specific biochemical properties (52).

Although additional studies are necessary, drugs with the ability to influence the interaction between both domains (and with the potential to eliminate the functional activity of ApoE4) have been proposed as pharmacological adjuvants to reduce this susceptibility to damage caused by intoxication with methylmercury and other neurodegenerative conditions (46, 47).

Different ApoE isoforms are also responsible for differences in the integrity of the blood-brain barrier in mouse models (53). Expression of ApoE4 but not ApoE2 and ApoE3 leads to a breakdown of the BBB following activation of the pro-inflammatory cytokine cyclophilin A (CypA) in pericytes, and causing activation of the NF-kB pathway and matrix metalloproteinases (53). Reinforcing this idea, a recent study on human subjects demonstrated that older individuals carrying ApoE4 showed higher values of the cerebrospinal fluid/plasma albumin quotient (a marker of BBB breakdown) than ApoE3 and/or ApoE2 carriers (54). Interestingly, this change in BBB integrity appears to precede cognitive decline and has been associated with high levels of CypA and active matrix metalloproteinase-9 (MMP-9), confirming the previous results in animals (54). Moreover, both CypA and MMP-9 levels are increased in pericytes and endothelial cells of the brains of AD patients carrying ApoE4, associated with accelerated pericyte degeneration and microvascular regression (55).

This breakdown of the integrity of the BBB found in ApoE4 individuals may permit the free entry of peripheral ApoE4 and mercury to the SNC, exacerbating the effects of these molecules in the intoxicated brain.

6. NEW PERSPECTIVES: OUR HYPOTHESIS

Although the exact molecular mechanism underlying the influence of ApoE4 in mercury intoxication is not fully understand, the association between the presence of the *epsilon* 4 allele and increased susceptibility to neurodegenerative consequences has been established (Table 2).

The presence of ApoE4 leads to numerous neuropathological effects (reviewed by (32) such as neurite growth impairment, cytoskeletal disarrangements, mitochondrial dysfunction (including altered membrane potential and decreased levels and activities of mitochondrial respiratory enzymes) and neuronal apoptosis. Recently, tau protein dysfunction, amyloid-beta deposition, impairment in BDNF maturation and production and neuroinflammation have also been observed (39, 56, 57).

In the CNS, mercury disturbs the oxidative balance and mitochondrial health (58), induces apoptosis, disrupts calcium homeostasis (59), causes the disarrangement of microtubules (60), reduces levels of antioxidant enzymes (61), provokes neuroinflammation (62), induces tau hyperphosphorylation (63) and the accumulation of amyloid-beta protein (64), and alters glutamate and γ-aminobutyric acid (GABA) signaling (65, 66).

Considering the numerous effects provoked in ApoE4 individuals intoxicated by mercury, only one mechanism (as that proposed by the theory of chelation by sulfhydryl groups of ApoE) is not enough to completely explain the correlation between the two factors.

Epidemiological studies on the genetic influence on the effects of mercury have demonstrated that the *APOE* gene shows consistent results related to neurodevelopment and neurotoxicity (toxicodynamic effects), but no studies have reported a possible influence on the toxicokinetics of a metal (see (67, 68) for reviews). Differently, the influence of the genes for glutathione and enzymes related to the synthesis and metabolism of gluthathione have been associated with alterations in the metal elimination (toxicokinetic effects) (67). Moreover, a post-mortem analysis of human brains recently revealed that mercury levels were not significantly correlated to the degree of brain neuropathology or ApoE isoforms (2). Considering the toxicokinetic characteristics of mercury (the half-life of mercury in the human body is approximately 70-80 days (69)), the quantification of mercury reveals exposure in recent months but does not provide information about previous exposures. So, to find no correlation between present levels of mercury and neuropathologic hallmarks is not surprising considering the long-term deleterious processes

associated with AD neuropathology. These data may indirectly support a more important influence of ApoE in mercury pharmacodynamics than in mercury pharmacokinetics.

Therefore, our hypothesis is that the presence of ApoE4 mainly affects toxicodynamic changes that act synergistically with the effects of mercury. Interestingly, individuals carrying the ApoE4 allele may already show many neuropathological changes with similar molecular mechanisms to those of neurotoxicity induced by mercury (Figure 3). There is the possibility of the facilitation of mercury-induced damage in the brains of ApoE4 individuals intoxicated with this metal. Based on the current evidence, synergic effects on pharmacodynamics between the two conditions (ApoE4 carriers and mercury intoxication) seems to be more probable and important that the effects caused by potentially disturbed clearance of mercury.

7. CONCLUSION

Based on the evidence described in this review and the neuropathological changes that the presence of ApoE4 and mercury neurotoxicity have in common, we propose a convergent action of both factors. The presence of ApoE4 seems to set the stage to potentiate the damage caused by mercury exposure. Increased knowledge of this interaction using epidemiological and pre-clinical studies is essential to improve prevention strategies and adequately manage intoxicated patients.

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