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Chapter 1: Review

Arsenic Neurotoxicity - A Review

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Abstract

Arsenic (As) is one of the oldest poisons known to men. Its applications throughout history are wide and varied: murder, make-up, paint and even as a pesticide. Chronic As toxicity is a global environmental health problem affecting millions of people in the Unites States and Germany to Bangladesh and Taiwan. Worldwide, As is released into the environment by melting of various metals, combustion of fossil fuels, as herbicides and fungicides in agricultural products. The drinking water in many countries, which is tapped from natural geological resources, is also contaminated as a result of the high level of arsenic in groundwater. The environmental fate of As is contamination of surface and groundwater with a contaminant level higher than 10 particles per billion (ppb) as set by World Health Organization (WHO). Arsenic exists in both organic and inorganic species and either form can also exist in a trivalent or pentavalent oxidation state. Long-term health effects of exposure to these As metabolites are severe and highly variable: skin and lung cancer, neurological effects, hypertension and cardiovascular diseases. Neurological effects of As may develop within a few hours after ingestion but usually is seen in 2-8 weeks after exposure. It is usually a symmetrical sensori-motor neuropathy, often resembling the Guillain-Barré syndrome. The predominant clinical features of neuropathy are paresthesias, numbness and pain, particularly in the soles of the feet. Electrophysiological studies performed on patients with As neuropathy have revealed a reduced nerve conducting velocity (NCV), typical of those seen in axonal degeneration. Most of the adverse effects of As are caused by inactivated enzymes in the cellular energy pathway, whereby As reacts with the thiol groups of proteins and enzymes and inhibits their catalytic activity. Furthermore, As-induced neurotoxicity, like many other neurodegenerative diseases, causes changes in cytoskeletal protein composition and hyperphosphorylation. These changes may lead to disorganization of the cytoskeletal framework, which is a potential mechanism of As-induced neurotoxicity.

Introduction

The word *arsenic* is derived from the Persian *zarnikh:* زرنیخ, meaning 'yellow orpiment'.[1] In Aramaic (זרניך) and Arabic (ألزرنيخ), the word was borrowed from the Persian. It came to the Western languages through the Greek rendering of *zarnikh:* 'αρσενικόν: *arsenikon,* which, in Greek, also means 'masculine'. Arsenic sulphide also occurs in a red form: *realgar* or *sandarach.* Long known and used in Persia and elsewhere since ancient times, As was also used in traditional Chinese and Indian medicine and as a cosmetic product in eye shadow in the Roman era. Given that the symptoms of acute As poisoning are easily confused with acute diarrhea associated with cholera, it quickly became a favorite homicidal agent. In 1832, a certain John Bodle was brought to trial for poisoning his grandfather by putting As in his coffee. James Marsh, a chemist working at the Royal Arsenal in Woolwich was called by the prosecution to detect its presence.[2] He performed the standard test by passing hydrogen sulfide through the suspect fluid. While Marsh was able to detect As, the yellow precipitate did not keep very well, and by the time it was presented to the jury it deteriorated. The jury was not convinced, and John Bodle was acquitted. Angered and frustrated by this, especially when John Bodle confessed later that he had indeed killed his grandfather, Marsh decided to devise a better test to demonstrate the presence of As. Taking Scheele's method as basis, he constructed a simple glass apparatus capable of not only detecting minute traces of As but also of measuring its quantity. While the Scheele test used nitric acid, in Marsh's case the suspect fluid would be mixed with sulfuric acid (H_2SO_4) and passed through a U-shaped tube with a piece of arsenic-free zinc at the end (Fig 1). Even a slight trace of As would cause arsine gas to form. When he ignited this gas, it decomposed into As and hydrogen and when he held a cold ceramic bowl against the flame, the As formed a silvery-black deposit mirror on the bowl. Not only could minute amounts of As be detected (as little as 0.02 mg), the test was very specific for As. Although antimony (Sb) could give a false-positive test by forming a similar black deposit, it would not react with sodium hypochlorite (NaOCl), while As would.[2,3]

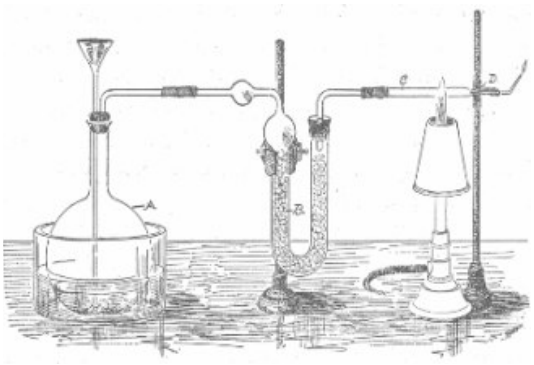

Fig 1. Schematic representation of the Marsh apparatus. (A) hydrogen generator, (B) chloride of calcium drying tube, (C) hard glass-tube and (D) Arsenic mirror.

Due to its use by the ruling class for killing one another and the incredible potency and discreetness, As has been called the Poison of Kings and the King of Poisons. The idea of using As as a murder weapon can even be seen in early movies and theater shows such as

Frank Capra's 'Arsenic and old lace', in which two elderly ladies used As in wine to kill their male suitors.

Arsenic has a long history of use as an intentional poison. Perhaps the most famous victim of As poisoning was Napoleon Bonaparte. He seems to have been exposed chronically during 1816, as appeared from neutron activation analysis of a hair sample.[4] A much lower concentration was found, however, in another hair sample attributed to Napoleon,[5] which may indicate that the identity of at least one of these samples should be questioned. One of the most prolific As poisoners in history was Goeie Mie ('Good Mary') of Leiden, The Netherlands, who lived in the 19th century. She poisoned at least 102 friends and relatives between 1867 and 1884, distributing arsenic-trioxide in hot milk to her victims after opening life insurance policies in their names. Of the 102 people poisoned, 45 persons became seriously ill, often with neurological symptoms, and 27 persons died; 16 of whom were her own relatives.[6]

Arsenic is used in folk medicine and in pesticides in many countries and also in modern western medicine for the treatment of leukemia.[7,8] In traditional Chinese medicine, preparations can be obtained in the form of coated or uncoated pills, powder or syrups. Different studies have shown that the majority of traditional Chinese medicines, such as Chinese herbal balls, show high doses of As varying between 0.1 and 36.6 mg per tablet, causing patients to get intoxicated by the high As dose, and Indian ayurvedic herbal medicine products are also known to cause lead, mercury and As intoxication.[9-12] Nowadays, the therapeutic use of As is making a comeback in modern medicine. Arsenic trioxide (ATO), for instance, is used to treat patients with relapsed acute promyelocytic leukemia (APL).[13-16] But ATO is also known for its less favorable side, as in causing temporary cardiac and neurotoxic side effects in APL patients.[8] Arsenic was one of the primary ingredients in pesticides before synthetic organic pesticides were available; its long-term application in agricultural pesticides has resulted in high levels of arsenic in the body of workers, who are exposed by inhalation during the spraying. Arsenic-containing rodent pesticides used for pest and insect control were banned due to human health concerns in production, use, and accidental poisoning and possible abuse in intentional poisoning. Various case reports and studies have revealed that exposure to As has resulted in various forms of cancer and peripheral neuropathy.[7,17,18]

Acute and chronic exposure to arsenic

Acute. A single exposure, to a high dose may lead to severe reactions such as diarrhea, vomiting, pain, dehydration and weakness. Nowadays, acute intoxication rarely occurs in western European countries; if it occurs, it is usually the result of intentional (suicide or homicide) or accidental poisoning. Occupational exposure to As is rare and usually occurs in the form of arsine gas, which causes symptoms different to those caused by As ingestion.[6] Exposure often occurs when arsine gas escapes during transport or when it is generated while arsenic-containing ores or metals are treated with acid.[19,20] Acute oral exposure to As is associated with gastrointestinal symptoms such as nausea, vomiting, abdominal pain and severe diarrhea. Cardiovascular and respiratory symptoms include hypotension, shock, pulmonary edema and heart failure. If survived, acute poisoning with As is also combined with neurological symptoms like light-headedness, weakness, delirium, encephalopathy and peripheral neuropathy, which have been reported.[21] Peripheral neuropathy as a result of As intoxication may be delayed several weeks after the initial toxic insult.[22] After a few weeks the patients show signs of recovery, however, when tested with electrophysiological studies 6 to 8 years after exposure, the patients still showed reduced motor conduction velocity.[22] Biopsies on the sural nerves 10 weeks after exposure showed axonal degeneration, which was at an early stage in some fibers. These findings suggest that diminished nerve velocity

conduction is a severe and latent effect of As intoxication, which contributes to As-induced neuropathy.

Chronic. Environmental and occupational As exposure is not only caused by contaminated drinking water due to the leaching of natural geological resources, but may also occur from mining and other industrial processes.[23]

Chronic ingestion of low concentration of As levels can occur through industrial accidents, work and environment, which eventually may cause a higher tolerance for As. An example of such an industrial accident can be found in the early 20th century, in reports on As intoxication caused by contaminated beer in Salford, UK.[24] The subject of these reports, the patients, had a few symptoms in common, they all suffered from 'peripheral neuritis' characterized by weakness in the limbs that made it difficult for them to walk[25]. In several cases some of the patients suffered from rashes and itching, sometimes these complaints were accompanied by darkening of skin texture. The presence of As in the beer was due to the use of contaminated glucose and inverted sugar by the brewers. Furthermore, the contamination of this sugar is caused by the use of very impure sulphuric acid. The source of As contamination was traced back to invert sugar, which was caused by the action of sulphuric acid on various forms of starch. The original source was the Spanish pyrites from which the sulphuric acid was made and often contained large percentages of As.

Chronic As poisoning represents a global, serious health concern, if As can be found in high concentrated levels in the environment from natural or industrial processes. Arsenic contamination of groundwater has occurred in various parts of the world such as in the Americas, Bangladesh, India, Taiwan and many other Asian countries. In Bangladesh, a country of 125 million, between 35-77 million people are currently exposed to As through drinking water, which is also the major cause of death, especially among children.[26,27] As a quick and inexpensive solution for the lack of sufficient and clean drinking water, UNICEF and the World Bank advocated to tap into deeper groundwater. Millions of wells were constructed; infant mortality and gastrointestinal illness were reduced by half. However, later studies revealed that over 40% of these wells are contaminated with arsenic. As a result, due to the daily exposure to As in their drinking water, the population in south east Asia has to endure various ailments caused by As. Chronic symptoms of As exposure are pigmentation changes, gastrointestinal symptoms, anemia, liver disease, a metallic taste and Mees' lines on the nails,[28] blackfoot disease and diabetes mellitus.[29] Apart from pigmentation changes, arsenic-induced skin pathology caused by chronic intoxication also causes hyperkeratosis, Bowen's disease, squamous cell carcinoma and basal cell carcinomas.[30,31] Hafemann *et al*. (2005) have shown an association between arsenic exposure and peripheral neuropathy in the Bangladesh population that has been chronically exposed to arsenic in drinking water. They showed that increased As exposure, as measured by both cumulative and urinary measures, was associated with evidence of sub-clinical sensory neuropathy. The correlation between As exposure and neuropathy was shown with the increased vibrotactile threshold, a sign of subclinical sensory neuropathy, on the one hand and sub-clinical loss of vibratory sensation in the lower extremities, on the other hand.[32]

Diagnosis and treatment of As poisoning

Arsenic concentration measurements for diagnostic purpose are usually carried out in urine. Acute As toxicity is usually diagnosed by increased urinary As in excess of 50 µg/l urine sample or 100 µg in 24-hour urine, and a shorter time span before examination, if no seafood has been ingested. The urine is collected in metal-free containers. Other biological samples, such as blood, and even hair and nails in chronic cases, are also used in the clinical laboratory.

For treatment of acute As poisoning, the primary concern is to correct the dehydration caused by As and restore vital bodily functions. In order to increase elimination, physicians prescribe gastric lavage and activated charcoal, but haemodialysis may also be considered. The efficacy of these detoxification methods, however, has not been well recorded. Although the metalloid As cannot be categorized as a metal, it shows some metal properties. Based on this fact, chelators can be used to remove As ions from the body. For treatment of acute As poisoning, the chelator 2.3-dimercapto-1-propanol (British Anti-lewisite, BAL) has been used with successful results.[33,34] Patients who were administered this drug showed elevated As excretion in their urine. At follow-up, their urinary As concentration was decreased to the background level. At the moment of admittance, neurological examination demonstrated no signs of nervous system depression. However, these patients survived the high dose of ingested As with only latent neuropathy symptoms. Neurological complications such as distal, symmetrical, sensory, axonal neuropathy are late effects of acute As poisoning. These neurological effects are non-responsive to chelation.[35] In clinical cases with chronically poisoned patients, trials with 4 As chelators such as BAL, meso-2.3-dimercaptosuccinic acid (DMSA), D-penicillamine and sodium 2.3-dimercapto-1-propamesulfonate (DMPS) did not provide any clinical, biochemical or histopathological benefits.[36,37] On the other hand studies done in rats with an As chelator such as BAL showed depletion of tissue As and its excretion via urine and faeces.[38] Although the binding affinity of a chelating agent for the metal is greater than for endogenous ligands, chelating is generally ineffective for treating established arsenical peripheral neuropathy.[39]

Kinetics of various arsenic metabolites and their molecular mechanism of toxicity

Kinetics. Arsenic absorption takes place mainly in the small intestines; also a minimal absorption occurs from skin contact and inhalation.[31,40,41] After ingestion, As is metabolized from inorganic to organic compounds with varying degrees of toxicity and the behavior of the different As species vary markedly. Arsenic speciation of inorganic and organic forms of As is often as important as total quantification, because of their different toxicity and mobility.[42] As speciation in biological samples is an essential tool to gain insight into its distribution in tissues and its specific toxicity to target organs.

Arsenic metabolites exist both in organic and inorganic forms and both types can exist in either trivalent or pentavalent oxidation states. The bioavailability of inorganic As is up to 60%. Inorganic As such as arsenate (iAs^{V}), the pentavalent form, and arsenite (iAs^{III}), the trivalent form, are the most aggressive single-substance toxicants, specially the trivalent form. A redox reaction reduces the pentavalent As to its trivalent state. This reduction step from pentavalent to trivalent, releases a more toxic compound, which in fact results in bio-activation (Fig. 2).

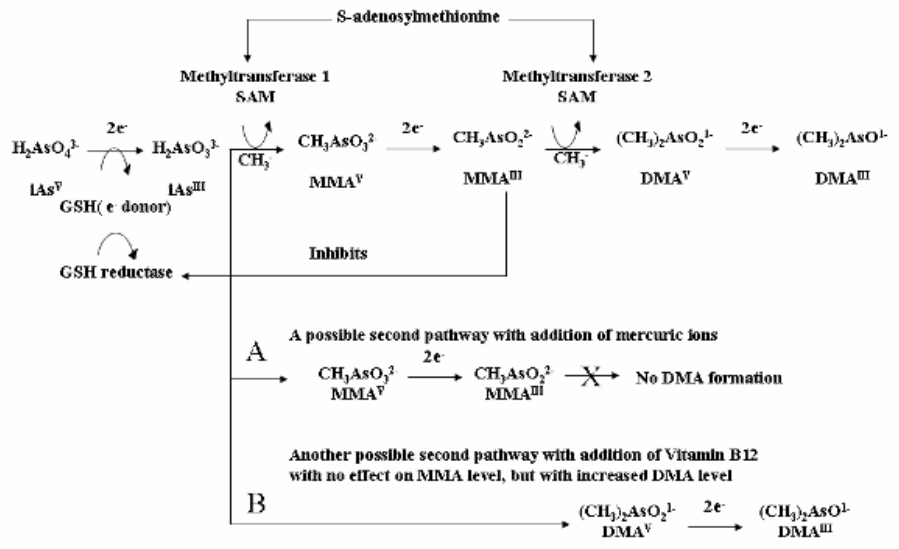

Fig. 2. Two possible pathways are shown for arsenate methylation. The main pathway is a straightforward bio-activation (metabolic activation) of arsenate in MMA, DMA and the theoretical conversion into TMA (not shown).[46,53,59] A possible second pathway is direct metabolism of iAs (A) to either MMA without further conversion to DMA or (B) increase in DMA levels, while the MMA levels stay intact.[44]

Arsenic metabolism shows a common route of absorption, distribution and excretion both in humans and various animals with subtle interspecies differences. Metabolism of inorganic As starts with intake and absorption. Distribution of As varies per species. This is a result of various factors such as species, bodyweight, route of intake and duration of exposure. After its absorption, As can be found in different organs, especially in the liver.[43,44] Arsenic undergoes hepatic biomethylation to form monomethyl arsenic (MMA) and dimethyl arsenic (DMA). Various *in vitro* studies on human and animal livers reveal the next step in iAs detoxification (Fig. 2). Detoxification starts with transformation of inorganic As to organic As and reduction of pentavalent arsenate to trivalent arsenite, which results in a more toxic component. The reduction of arsenate to arsenite is catalyzed by gluthatione (GSH) and other thiols, which are reducing agents.[45] Arsenate at physiological pH is ionized and as such is not able to pass cellular membranes. Conversion to arsenite at physiological pH facilitates passage through the cellular membrane.

Metabolism of As continues by using arsenite as its substrate for methylation, which is distributed in tissues and cytosol. However, methylation does not appear to be the primary detoxification pathway for arsenite. Protein binding has been suggested as the initial protective mechanism. Arsenite binding to protein serves as a reservoir and takes place after initial increase in arsenite concentration. When methylation enzymes start to become effective, the reservoir may slowly release small amounts of arsenite for methylation.[46] The methylation of arsenite is catalyzed by a specific methyltransferase using s-adenosylmethionine as a methyl donating cofactor (SAM).[43,47] Addition of a methyl group to arsenite leads to synthesis of pentavalent monomethylarsonic (MMA^V). The MMA^V in turn is reduced to trivalent MMA (MMA^{III}) by GSH, the reducing agent. Another round of methyltransferase activity with MMA^{III} results in production of dimethylarsenic acid (DMA).

However, it is not clear whether the same SAM is used for methylation of MMA and DMA or that two separate SAMs are being used for these two methylation steps. Theoretically, conversion of arsenate to DMA could be extended by one more round to achieve trimethylarsenic acid (TMA).[43,48] A second possible conversion route to MMA and DMA was introduced by the *in vitro* studies of Buchet and Lauwerys, by adding mercuric ions; they prevented the formation of DMA without affecting MMA formation.[44, 45] Furthermore, it was evident that the addition of cyanocobalamin or methylcyanocobalamin (vitamin B12) and coenzyme B12 in combination with SAM resulted in a significant increase of DMA and no additional increase in MMA level. This experiment revealed two different enzymatic activities involved in methytransferase to either MMA or DMA (Fig 2).

The methylation process has been thought to be the detoxification mechanism for As. This is true when this line of reasoning is applied to parameters of acute toxicity of As species, such as LC_{50} or LD_{50}. As a rule, the trivalent As compounds are more cytotoxic then their pentavalent forms. However, various studies in animals and cell cultures have shown the adverse effects of methylated As, such as DMA^V as a tumor promoter[49,50] or direct genotoxic action of MMA^{III} and DMA^{III} *in vitro*.[43,51]

The main excretion route of As is through the urine and bile. However, the various As metabolites do not excrete in the same fashion in different animals and humans. For example, studies in rats have shown that pentavalent metabolites such as iAs^V, MMA^V and DMA^V are exclusively excreted into urine, MMA^{III} only into bile and iAs^{III} into both bile and urine.[52] In contrast, human studies revealed the presence of MMA^{III} in urine.[53,54] Arsenic studies carried out by Csanaky and Gregus in rats, mice, hamsters, rabbits and guinea pigs revealed some similarities. All species injected with iAs^V excreted various As metabolites into urine, in contrast to injection with iAs^{III}, which showed higher excretion into bile rather than urine.[52]

Mechanism. These metabolites exert their toxicity by inactivating many enzymes, especially those involved in the cellular energy pathway and DNA synthesis and repair. Arsenic is substituted for phosphate in high-energy compounds such as ATP. Arsenic binds covalently with sulfhydryl groups in their reduced form. These interactions also take place with certain enzymes necessary for cellular metabolism.[17] Various As carcinogenesis studies have revealed that As may alter one or more DNA repair processes. Andrew *et al*. have shown that patients exposed to As have altered nucleotide excision repair mainly on the excision repair cross-complementing 1 (ERCC1) component. Arsenic exposure was associated with decreased expression of ERCC1 in isolated lymphocytes at the mRNA and protein levels.[55] In short, As exposure results not only in general toxicity but also in neuronal diseases and carcinogenesis.

Tri-valent arsenic (iAs^{III}, MMA^{III} and DMA^{III}) compounds are thought to interact with thiol groups of proteins and enzymes in their reduced state. This is believed to inhibit the catalytic activity of enzymes.[54] The common thinking is that tri-valent arsenic metabolites inhibit pyruvate dehydrogenase (PDH), which leads to disruption of the energy system of the cell,[56] which in turn may release an apoptosis-inducing factor (AIF) resulting in cell damage and death. AIF is released from the mitochondrial intermembrane space from where it translocates to the cell nucleus.[57] Apoptosis is associated with early formation of ring-like perinuclear condensed chromatin co-localized with AIF, DNA fragmentation and finally cell death. Pentavalent As (iAs^V, MMA^V and DMA^V) is substituted for phosphorus in many biochemical reactions. Replacing the stable phosphorus anion in phosphate with the less stable As^V anion leads to rapid hydrolysis of high-energy bonds in compounds such as ATP. At the level of the citric acid cycle, As inhibits succinate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. This leads to loss of high-energy phosphate bonds and effectively uncouples oxidative phosphorylation.[58,59]

Another approach is that trivalent As inhibits enzyme complexes by reactive oxygen species (ROS), indicating that ROS production by trivalent As causes pyruvate dehydrogenase (PDH) inactivation through oxidation.[60] Inactivation through oxidation causes impaired gluconeogenesis and reduced oxidative phosphorylation.[61] Production of ROS by As was determined by use of a nonfluorescent dye 5',6'-chlomethyl-2',7'-dichlorodihydrofluorscein (CM-H_2DCFDA), which is a non-specific radical detector to identify the radical species.[62] Cells pretreated with CM-H_2DCFDA and subsequently exposed to arsenite exhibit a dose-dependent increase in fluorescence levels within minutes of treatment when compared to controls.[63] Using DMSO in these experiments as radical scavenger in the reaction mix reduced the fluorescence signal to a near-background level.

Arsenic-induced neurotoxicity

Arsenic effects manifest themselves weeks after first exposure as both central and peripheral neuropathy. Central neuropathy due to As poisoning has been reported to cause impairment to neurological functions such as learning, short-term memory and concentration.[64] People chronically poisoned by arsenic occurring naturally in groundwater may suffer from toxic delirium and encephalopathy.[39] Neuropsychological tests showed mildly impaired psychomotor speed and attentive processes, whereas verbal learning and memory were severely impaired.
The most frequent neurological manifestation by As is peripheral neuropathy that may last for several years or even life-long. The peripheral neuropathy may lead to rapid severe ascending weakness, similar to the Guillan-Barré syndrome, requiring mechanical ventilation. Peripheral neuropathy is common in persons chronically exposed to As-contaminated drinking water.[65]
From human clinical cases studied by Le Quesne and McLeod it has become clear that As exposure results in a latent reaction to the nervous system, which was established through their reduced Nerve Conduction Velocities (NCVs) measurements.[22] These patients showed some recovery in the years following exposure to As; however, a full NCV regain was not achieved. It is doubtful whether PNS symptoms will ever disappear completely.
Patients exposed to As show significantly lower NCVs in their peripheral nerves in comparison to their referents.[21,34,66] Perhaps, changes in cytoskeletal composition may be the major reason of As poisoning leading to axonal degeneration, which in turn could lead to axonopathy.
An actual mechanistic model for arsenic neurotoxicity is as yet not easy to hypothesize, although interference with cytoskeletal proteins is a primary consideration. Therefore, one may look for such mechanistic parallels in other neurotoxins, especially neurotoxic metals, but also in other neurodegenerative diseases.

Metals as environmental pollutants such as lead and mercury have been associated with neurodegenerative diseases.[67] Exposure to aluminum, lead and mercury are known to have caused abnormalities in the nervous system related to interference with the cytoskeleton. Clinically, symptoms may occur as peripheral and central neuropathies. Aluminum has been known to cause dialysis encephalopathy in some individuals with renal failure. Furthermore, it has been suggested that aluminum might be implicated in Alzheimer's disease (AD), because of some similarities in pathological changes. The pathological changes in both cases can be summarized as presence of neurofibrillary tangles as diagnostic hallmarks AD.[68] However, further examination of these tangles has shown differences in their tangles between AD and the aluminum-exposed patients, in AD patients tangles consist of paired helical filaments, whereas those induced by aluminum are single.[69] Aluminum exposure in animal studies has shown induction of neurofibrillary degeneration. Phosphorylation of cytoskeletal proteins appears to modulate their interactions with one another and with other cellular

proteins. Disruption of the phosphorylation of cytoskeletal proteins results in disorganization of the cytoskeletal structure.[70] Other metals as neurotoxicants such as lead have also been indicated in the etiology of amyotrophic lateral sclerosis (ALS), whereas manganese has been reported to be involved in Parkinson's disease (PD) or a similar syndrome, Parkinsonism. Exposure to lead has been shown to be related to ALS in a case control study conducted in New England from 1993 to 1996.[71,72] Generally, ALS is divided into two forms: (1) the classic sporadic form; (2) the familial, presumably hereditary form. The cause of the sporadic form is unknown. The cause of the familial form is believed to be genetic, attributable to a mutation in Cu–Zn superoxide dismutase.[73] Studies in occupational exposure have suggested an association between PD and elevated exposure to manganese.[74] Exposure to manganese can cause neurotoxicity and a neurological syndrome that resembles PD.[75]

Neurofilament proteins are major constitutes of neurons and they control axonal caliber, transport and signal.[76] In neurodegenerative diseases such as AD it appears that the metabolism of neurofilaments is disturbed, as indicated by the presence of neurofilament epitopes in the neurofibrillary tangles, as well as by the severe reduction of the expression of the gene for the light neurofilament subunit of the neurofilament triplet (neurofilament High, -Middle and –Light) in brains of AD patients.[77] Accumulation of neurofilaments in the proximal cell body and the perikaryon of motor neurons is a hallmark of ALS and PD.[73,76] Disruption and disorganization of neurofilament transport and neuron cytoskeletal network is a pathological feature seen in all of these neurodegenerative diseases.

In rats exposed to As , decrease of the neurofilament Light subunit (NF-L) in sciatic nerve is evident.[78] Arsenic-induced decrease of NF-L may play an important role in the pathological changes of the nervous system, since NF-L is the only NF protein capable of independently organizing and co-assembling filaments *in vivo*. Both NF-H and NF-M need NF-L protein to form a heteropolymer in the cytoskeletal framework.[79] However, in vitro studies with iAs^{III} in neuroblastoma (SK-N-SH) and Schwannoma (ST-8814) cell lines show no effect on their mRNA expression level of cytoskeletal genes.[80] Thus, it can be suggested that the decrease in NF-L expression is a post-translational activity as a result of a proteolytic process. Calpain (calcium-activated cytoplasmic protease) could be responsible for NF-L degradation, since neuroblastoma cells (SY-5Y) treated with arsenic trioxide (trivalent As) show an increase in intracellular calcium.[81] Studies in PC12 cells under oxidative stress circumstances have shown an increase of calcium in the cells and up-regulation of calpain leading to degradation of NF-L protein.[82] Furthermore, inactivation of calpain by calpain inhibitor (MDL-28170) prevents NF-L breakdown.[83,84] These results suggest that As-induced destabilization and disruption of the cytoskeletal framework is partly due to activation of calpain, through influx of Ca^{2+}, which in turn is responsible for NF-L degradation in a calcium-induced proteolytic process.

Another important cytoskeletal protein in neurodegenerative diseases is the tau protein (MAP-tau), which is a member of the microtubule protein family transcripted by alternative splicing of a single gene. It has tandem repeats of a tubulin binding domain and promotes tubulin assembly. Although tau proteins are found in all cells, they are major components of neurons where they are predominantly associated with microtubules of the axon. Changes in tau-protein may play a role in the pathogenesis of neurodegenerative diseases. In AD patients, MAP-tau becomes abnormally hyperphosphorylated and accumulates as tangles of paired helical filaments in neurons undergoing degeneration. Hyperphosphorylated MAP-tau disorganizes microtubules assembly from normal tau and tubulin, which may lead to the formation of the neurofibrillary tangles and the degeneration of the affected neurons in AD patients. Arsenic may affect the phosphorylation of tau-proteins as well. Giasson *et al.* demonstrated hyperphosphorylation of tau-proteins in Chinese hamster ovary (CHO) cells *in vitro* after treatment with iAs^{III}.[85] They also showed that iAs^{III} causes a significant increase in

the phosphorylation of several amino acid residues in tau. This is in line with experiments in rats with iAs^{III}, which have also shown that the rats' MAP-tau was hyperphosphorylated after dosing them with iAs^{III} {unpublished data}. These results indicate that As may be involved in the cascade leading to deregulation of tau function associated with neurodegeneration.

Conclusion

Arsenic compounds are toxic substances with very profound effects on human health. Metabolism of As involves reduction of pentavalent As to a trivalent state and subsequent oxidative methylation. The adverse effects caused by As metabolites vary. The molecular mechanism of action can be summarized as involvement in cellular energy pathway, disruption of oxidative phosphorylation, hyperphosphorylation and altering the DNA synthesis and repair. Acute As intoxication has only been treated successfully with BAL. Furthermore, none of the chelators have ever been successful in treating chronic As intoxication. Arsenic neuropathy occurs weeks or even months after initial exposure in the acute cases, and it is also present in chronically exposed patients. Reduced nerve conducting velocity in PNS is a hallmark of As neuropathy. The neuropathy is primarily due to destruction of axonal cylinders and compositional changes, leading to axonopathy. The cytoskeletal changes are caused by disruption of the neurofilament and microtubule network in the nerve cells, namely through gradual degradation of NF-L by calpain, since iAs^{III} does not affect expression on gene level and hyperphosphorylation of NF-L and MAP-tau. The probable functional disturbances of these proteins such as transport and signaling and pathological changes such as reduction in caliber of axons may lead to axonopathy. These functional and pathological changes manifest themselves in clinical signs of neuropathy.

Reference List

1 Steingass A. A Comprehensive Persian English Dictionary, Kegan Paul, Trench, Trubner & co., LTD., London 1947.

2 Marsh J. Account of a method of separating small quantities of arsenic from substances with which it may be mixed. *Eddinburgh New Philosophical Journal* 1836; **21**: 229-36.

3 Wikipedia. Arsenic. Wikipedia . 2007. Ref Type: Electronic Citation

4 Leslie AC, Smith H. Napoleon Bonaparte's exposure to arsenic during 1816. *Arch Toxicol* 1978; **41**: 163-67.

5 Lewin PK, Hancock RG, Voynovich P. Napoleon Bonaparte--no evidence of chronic arsenic poisoning. *Nature* 1982; **229**: 627-28.

6 De Wolff FA, Edelbroek PM. Neurotoxicity of arsenic and its compounds. In *Handbook of Clinical Neurology* ed. by Vinken & Bruyn's, Elsevier Science B.V. 1994; 283-291.

7 Dich J, Zahm SH, Hanberg A, Adami HO. Pesticides and cancer. *Cancer Causes Control* 1997; **8**: 420-43.

8 Yip SF, Yeung YM, Tsui EY. Severe neurotoxicity following arsenic therapy for acute promyelocytic leukemia: potentiation by thiamine deficiency. *Blood* 2002; **99**: 3481-82.

9 Espinoza EO, Mann MJ, Bleasdell B. Arsenic and mercury in traditional Chinese herbal balls. *N Engl J Med* 1995; **333**: 803-804.

10 Ong ES, Yong YL, Woo SO. Determination of arsenic in traditional Chinese medicine by microwave digestion with flow injection-inductively coupled plasma mass spectrometry (FI-ICP-MS). *J AOAC Int* 1999; **82**: 963-67.

11 Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, Phillips RS. Heavy metal content of ayurvedic herbal medicine products. *JAMA* 2004; **292**: 2868-73.

12 Lynch E, Braithwaite R. A review of the clinical and toxicological aspects of 'traditional' (herbal) medicines adulterated with heavy metals. *Expert Opin Drug Saf* 2005; **4**: 769-78.

13 Jing Y, Wang L, Xia L, Chen GQ, Chen Z, Miller WH, Waxman S. Combined effect of all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia cells in vitro and in vivo. *Blood* 2001; **97:** 264-69.

14 Shen Y, Shen ZX, Yan H, Chen J, Zeng XY, Li JM, Li XS, Wu W, Xiong SM, Zhao WL, Tang W, Wu F, Liu YF, Niu C, Wang ZY, Chen SJ, Chen Z. Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. *Leukemia* 2001; **15**: 735-41.

15 Miller Jr WH, Schipper HM, Lee JS, Singer J, Waxman S, Mechanisms of action of arsenic trioxide. *Cancer Res* 2002; **62**: 3893-3903.

16 Tallman MS. Treatment of relapsed or refractory acute promyelocytic leukemia. *Best Pract Res Clin Haematol* 2007; **20**: 57-65.

17 Brouwer OF, Onkenhout W, Edelbroek PM, de Kom JF, de Wolff FA, Peters AC, Increased neurotoxicity of arsenic in methylenetetrahydrofolate reductase deficiency. *Clin Neurol Neurosur* 1992;**94**: 307-310.

18 Heaven R, Duncan M, Vukelja SJ. Arsenic intoxication presenting with macrocytosis and peripheral neuropathy, without anemia. *Acta Haematol* 1994; **92**: 142-43.

19 Wilkinson SP, McHugh P, Horsley S, Tubbs H, Lewis M, Thould A, Winterton M, Parsons V, Williams R. Arsine toxicity aboard the Asiafreighter. *Br Med J* 1975; **3**: 559-563.

20 Pullen-James S, Woods SE. Occupational arsine gas exposure. *J Natl Med Assoc* 2006; **98**: 1998-2001.

21 Greenberg SA. Acute demyelinating polyneuropathy with arsenic ingestion. *Muscle Nerve* 1996; **19**: 1611-13.

22 Le Quesne PM, McLeod JG. Peripheral neuropathy following a single exposure to arsenic. Clincal course in four patients with electrophysiological and histological studies. *J Neurol Sci* 1977; **32**: 437-51.

23 Centeno JA, Tseng CH, van der Voet GB, Finkelman RB. Global impacts of geogenic arsenic: a medical geology research case. *Ambio.* 2007; **36**: 78-81.

24 Kelynack TNV. Lond MRCD. Arsenical poisoning from beer drinking. *The Lancet* 1900;1600-1603.

25 Buchanan RJM, Lond MRCD. Cases of arsenical peripheral neuritis. *The Lancet* 1901; 170-72.

26 Mayans MV, Robertson SE, Duclos P. Adverse events monitoring as a routine component of vaccine clinical trials: evidence from the WHO Vaccine Trial Registry. *Bull World Health Organ* 2000; **78**: 1167.

27 Mukherjee A, Sengupta MK, Hossain MA, Ahamed S, Das B, Nayak B, Lodh D, Rahman MM, Chakraborti D. Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. *J Health Popul Nutr* 2006; **24**: 142-63.

28 Mees RA. Een verschijnsel bij polyneuritis arsenicosa. *Ned Tijdsch Geneeskd* 1919; **1**: 391-96.

29 Chen C.J, Wang SL, Chiou JM, Tseng CH, Chiou HY, Hsueh YM, Chen SY, Wu MM, Lai MS. Arsenic and diabetes and hypertension in human populations: A review. *Toxicol Appl Pharmacol* 2007.

30 Chowdhury UK, Biswas BK, Chowdhury TR, Samanta G, Mandal BK, Basu GC, Chanda CR, Lodh D, Saha KC, Mukherjee SK, Roy S, Kabir S, Quamruzzaman Q, Chakraborti D. Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect* 2000; **108**: 393-97.

31 Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, Thompson C, Ladich ER. Pathology related to chronic arsenic exposure. *Environ. Health Perspect* 2002; **110**: 883-86.

32 Hafeman DM, Ahsan H, Louis ED, Siddique AB, Slavkovich V, Cheng Z, van Geen A, Graziano JH. Association between arsenic exposure and a measure of subclinical sensory neuropathy in Bangladesh. *J Occup Environ Med* 2005; **47**: 778-84.

33 Vantroyen B, Heilier JF, Meulemans A, Michels A, Buchet JP, Vanderschueren S, Haufroid V, Sabbe M. Survival after a lethal dose of arsenic trioxide. *J Toxicol Clin Toxicol* 2004; **42**: 889-95.

34 Tseng HP, Wang YH, Wu MM, The HW, Chiou HY, Chen CJ. Association between chronic exposure to arsenic and slow nerve conduction velocity among adolescents in Taiwan. *J Health Popul Nutr* 2006; **24**: 182-89.

35 Perriol MP, Devos D, Hurtevent JF, Tiffreau V, Saulnier F, Destee A, Defebvre L. A case of neuropathy mimicking Guillain-Barre syndrome after arsenic intoxication. *Rev Neurol* 2006; **162**: 374-77.

36 Rahman MM, Chowdhury UK, Mukherjee SC, Mondal BK, Paul K, Lodh D, Biswas BK, Chanda CR, Basu GK, Saha KC, Roy S, Das R, Palit SK, Quamruzzaman Q, Chakraborti D. Chronic arsenic toxicity

in Bangladesh and West Bengal, India--a review and commentary. *J Toxicol Clin Toxicol* 2001; **39**: 683-700.

37 Stenehjem AE, Vahter M, Nermell B, Aasen J, Lierhagen S, Morland J, Jacobsen D. Slow recovery from severe inorganic arsenic poisoning despite treatment with DMSA (2.3-dimercaptosuccinic acid). *Clin Toxicol* 2007; **45**: 424-28.

38 Hilmy AM, el Domiaty NA, Kamal MA, Mohamed MA, Abou Samra WE. Effect of some arsenic antagonists on the toxicity, distribution and excretion of arsenite and arsenate in rats. *Comp Biochem Physiol C* 1991; **99**: 357-62.

39 Hall AH. Chronic arsenic poisoning. *Toxicol Lett* 2002; **128**: 69-72.

40 Enterline PE, Henderson VL, Marsh GM. Exposure to arsenic and respiratory cancer. A reanalysis. *Am J Epidemiol* 1987; **125**: 929-38.

41 Hertz-Picciotto I, Smith AH. Observations on the dose-response curve for arsenic exposure and lung cancer. *Scand. J Work Environ Health* 1993; **19**: 217-26.

42 Mandal BK, Ogra Y. Anzai K, Suzuki KT. Speciation of arsenic in biological samples. *Toxicol Appl Pharmacol* 2004; **198**; 307-318.

43 Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch Toxicol* 2000; **74**: 289-99.

44 Buchet JP, Lauwerys R. Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. *Arch Toxicol* 1985; **57**: 125-29.

45 Buchet JP, Lauwerys R. Role of thiols in the in-vitro methylation of inorganic arsenic by rat liver cytosol. *Biochem Pharmacol* 1988; **37**: 3149-53.

46 Aposhian HV. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu Rev Pharmacol Toxicol* 1997; **37**: 397-419.

47 Shirachi DY, Lakso JU, Rose LJ. Methylation of sodium arsenate by rat liver in vitro. *Proc West Pharmacol Soc* 1981; **24**: 159-60.

48 Kitagawa F, Shiomi K, Otsuka K. Analysis of arsenic compounds by capillary electrophoresis using indirect UV and mass spectrometric detections. *Electrophoresis* 2006; **27**: 2233-39.

49 Yamamoto S, Konishi Y, Matsuda T, Murai T, Shibata MA, Matsui-Yuasa I, Otani S, Kuroda K, Endo G, Fukushima S. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. *Cancer Res* 1995; **55**: 1271-76.

50 Wanibuchi H, Yamamoto S, Chen H, Yoshida K, Endo G, Hori T, Fukushima S. Promoting effects of dimethylarsinic acid on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. *Carcinogenesis* 1996; **17**: 2435-39.

51 Colognato R, Coppede F, Ponti J, Sabbioni E, Migliore L. Genotoxicity induced by arsenic compounds in peripheral human lymphocytes analysed by cytokinesis-block micronucleus assay. *Mutagenesis* 2007.

52 Csanaky I, Gregus Z. Species variations in the biliary and urinary excretion of arsenate, arsenite and their metabolites. *Comp Biochem Physiol C Toxicol Pharmacol* 2002; **131:** 355-65.

53 Vahter M, Concha G. Role of metabolism in arsenic toxicity. *Pharmacol Toxicol* 2001; **89**: 1-5.

54 Aposhian HV, Zakharyan RA, Avram MD, Sampayo-Reyes A, Wollenberg ML. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxication of the trivalent arsenic species. *Toxicol Appl Pharmacol* 2004; **198**: 327-35.

55 Andrew AS, Burgess JL, Meza MM, Demidenko E, Waugh MG, Hamilton JW, Karagas MR. Arsenic exposure is associated with decreased DNA repair in vitro and in individuals exposed to drinking water arsenic. *Environ Health Perspect* 2006; **114**: 1193-98.

56 Aposhian HV, Aposhian MM. Arsenic toxicology: five questions. *Chem Res Toxicol* 2006; **19**: 1-15.

57 Akay C, Thomas III C, Gazitt Y. Arsenic trioxide and paclitaxel induce apoptosis by different mechanisms. *Cell Cycle* 2004; **3**: 324-34.

58 Saha JC, Dikshit AK, Bandyopadhyay M, Saha KC. A Review of Arsenic Poisoning and its Effects on Human Health. *Critical Reviews in Environmental Science and Technology,* 1999; **29**: 281-313.

59 Hughes MF. Arsenic toxicity and potential mechanisms of action. *Toxicol Lett* 2002; **133**: 1-16.

60 Samikkannu T, Chen CH, Yih LH, Wang AS, Lin SY, Chen TC, Jan KY. Reactive oxygen species are involved in arsenic trioxide inhibition of pyruvate dehydrogenase activity. *Chem Res Toxicol* 2003; **16**: 409-414.

61 Szinicz L, Forth W. Effect of As2O3 on gluconeogenesis. *Arch Toxicol* 1988; **61**: 444-49.

62 LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem Res Toxicol* 1992; **5**: 227-31.

63 Liu SX, Athar M, Lippai I, Waldren C, Hei TK. Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. *Proc Natl Acad Sci USA* 2001; **98**: 1643-48.

64 Bolla-Wilson K, Bleecker and ML. Neuropsychological impairment following inorganic arsenic exposure. *J Occup Med* 1987; **29**: 500-503.

65 Mazumder DN, Das Gupta J, Chakraborty AK, Chatterjee A, Das D, Chakraborti D. Environmental pollution and chronic arsenicosis in south Calcutta. *Bull World Health Organ* 1992; **70**: 481-85.

66 Otto D, Xia Y, Li Y, Wu K, He L, Telech J, Hundell H, Prah J, Mumford J, Wade T. Neurosensory effects of chronic human exposure to arsenic associated with body burden and environmental measures. *Hum Exp Toxicol* 2007; **26**: 169-77.

67 Monnet-Tschudi F, Zurich MG, Boschat C, Corbaz A, Honegger P. Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev Environ Health* 2006; **21**: 105-117.

68 van der Voet GB, Marani E, Tio S, De Wolff and FA, Aluminum neurotoxicity. In *Prog Histochem Cytochem* ed. by W. Graumann and J. Gustav Fischer Verlag, Stuttgart; New York 1991; 235-42.

69 Shin RW, Lee VM, Trojanowski JQ. Neurofibrillary pathology and aluminum in Alzheimer's disease. *Histol Histopathol* 1995; **10**: 969-78.

70 Kaur A, Joshi K, Minz RW, Gill KD, Neurofilament phosphorylation and disruption: a possible mechanism of chronic aluminium toxicity in Wistar rats. *Toxicology* 2006; **219**: 1-10.

71 Kamel F, Umbach DM, Munsat TL, Shefner JM, Hu H, Sandler DP. Lead exposure and amyotrophic lateral sclerosis. *Epidemiology* 2002; **13**: 311-19.

72 Kamel F, Umbach DM, Hu H, Munsat TL, Shefner JM, Taylor JA, Sandler DP. Lead exposure as a risk factor for amyotrophic lateral sclerosis. *Neurodegener Dis* 2005; **2**: 195-201.

73 Al Chalabi A, Leigh PN. Recent advances in amyotrophic lateral sclerosis. *Curr Opin Neurol* 2000; **13**: 397-405.

74 Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, Koller W, Bowler RP, Mergler D, Bouchard M, Smith D, Gwiazda R, Doty RL. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. *Occup Environ Med* 2007; **64**: 167-77.

75 Yokel RA. Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration. *J Alzheimers Dis* 2006; **10**: 223-53.

76 Al Chalabi A, Miller CC. Neurofilaments and neurological disease. *Bioessays* 2003; **25**: 346-55.

77 Lacoste-Royal G, Mathieu M, Nalbantoglu J, Julien JP, Gauthier S, Gauvreau and D. Lack of association between two restriction fragment length polymorphisms in the genes for the light and heavy neurofilament proteins and Alzheimer's disease. *Can J Neurol Sci* 1990; **17**: 302-305.

78 Vahidnia A, Romijn F, Tiller M, v. d. Voet GB, De Wolff FA, Arsenic-Induced Toxicity: Effect on Protein Composition in Sciatic Nerve. *Hum Exp Toxicol* 2006; **25**: 667-74.

79 Carpenter DA, Ip W. Neurofilament triplet protein interactions: evidence for the preferred formation of NF-L-containing dimers and a putative function for the end domains. *J Cell Sci* 1996; **109**: 2493-98.

80 Vahidnia A, van der Straaten RJHM, Romijn F. van Pelt J, van der Voet GB, De Wolff and FA. Arsenic metabolites affect expression of the neurofilament and tau genes: An in-vitro study into the mechanism of arsenic neurotoxicity. Toxicol In Vitro . 2007. In Press. doi:10.1016/j.tiv.2007.04.007

81 Florea AM, Splettstoesser F, Büsselberg D. Arsenic trioxide (As2O3) induced calcium signals and cytotoxicity in two human cell lines: SY-5Y neuroblastoma and 293 embryonic kidney (HEK). *Toxicol Appl Pharmacol* 2007; **220**: 292-301.

82 Ray SK, Fidan M, Nowak MW, Wilford GG, Hogan EL, Banik NL. Oxidative stress and Ca2+ influx upregulate calpain and induce apoptosis in PC12 cells. *Brain Res* 2000; **852**: 326-34.

83 Kunz S, Niederberger E, Ehnert C, Coste O, Pfenninger A, Kruip J, Wendrich TM, Schmidtko A, Tegeder I, Geisslinger G. The calpain inhibitor MDL 28170 prevents inflammation-induced neurofilament light chain breakdown in the spinal cord and reduces thermal hyperalgesia. *Pain* 2004; 409-418.

84 Lopez-Picon FR, Kukko-Lukjanov TK, Holopainen IE. The calpain inhibitor MDL-28170 and the AMPA/KA receptor antagonist CNQX inhibit neurofilament degradation and enhance neuronal survival in kainic acid-treated hippocampal slice cultures. *Eur J Neurosci* 2006; **23**: 2686-94.

85 Giasson BI, Sampathu DM, Wilson CA, Vogelsberg-Ragaglia V, Mushynski WE, Lee and VM, The environmental toxin arsenite induces tau hyperphosphorylation. *Biochemistry* 2002; **41**: 15376-87.