Abstract

Arsenic toxicity has emerged as a global concern of prevalence especially in various Asian regions, highlighted with 130 million population at risk in India and Bangladesh. Arsenic toxicity has been associated with numerous health effects affecting almost every organ system. These adverse effects have been identified to establish or facilitate various diseased manifestations and pathological conditions that may be as severe as internal cancers. Despite the extensive research in the filed after the recognition of severity of the problem no ideal therapy is available for arsenicosis patients. Although toxicokinetic, especially the metabolism that plays crucial role in toxic advent along with molecular mechanism of arsenic toxicity is much investigated, we are far from providing clinical solution. Chelation therapy, which is recommended as the prime line of treatment in heavy metal toxicity has been proven ineffective or inappropriate due to adverse effect limitations. Development of new drug and newer therapeutic concepts suggested by handful of research groups working in the filed provide some hope for the clinical cases of millions of arsenic poisoning patients. In the present review we have highlighted and addressed in brief arsenic-induced pathological signs and symptoms, clinical diagnosis and available therapeutic solutions and relevant hypothesis.

Keywords: Arsenic, Methylation, Oxidative Stress, hyperkeratosis, chelation therapy, DMSA, Antioxidants

Introduction

Arsenic is one of the leading cause and concern of mass poisoning in children and adults in various parts of Asia especially Bangladesh and India (1, 2). It is posted as number 1 on the Agency for Toxic Substances and Disease Registry’s (ATSDR) “Top 20 List”. More than 20 arsenic compounds are present in the natural environment and biological systems. Trivalent arsenic species, such as inorganic arsenite (AsIII), monomethylarsonous acid (MMAIII), and dimethylarsinous acid (DMAIII) are more toxic compared to pentavalent arsenic species (AsV) (3). In general, the toxicity of arsenic compounds is in the following order: arsine >arsenites> arsenates > organic arsenicals > elemental arsenic. Inorganic arsenic compounds, which are found throughout the environment, can cause acute and chronic toxic effects. Human may encounter arsenic in contaminated drinking water from wells drilled into arsenic rich ground strata or in water contaminated by industrial or agro chemical waste. Exposure via drinking water has been associated with cancer of the skin and various internal organs as well as hyperkeratosis, pigmentation changes and effects on the circulatory and nervous system. Chronic arsenic toxicity due to drinking of arsenic contaminated water has been reported from many countries. Recently, large population in West Bengal in India and Bangladesh has reported to be affected with arsenic. The delayed health effects of exposure to arsenic, lack of common definitions and of local awareness as well as poor reporting in affected
areas are the major problems in determining the risk analysis and extent of the arsenic-in-drinking-water (4-6). Nearly 16 districts of West Bengal have been reported to have ground water arsenic concentrations above 0.05 mg/L where the WHO permissible limit amount to not more than 10ppb (7). Up to 90% of the Bangladesh population of 130 million drinks well water contained with high concentrations of arsenic. Piped water supplies are available only to a little more than 10% of the total population living in the large agglomerations and some district towns. The impact of arsenic extends from immediate health effect to extensive social and economic hardship.

**Absorption, Distribution, Metabolism and Excretion:** About 60-90% of soluble arsenic compounds are absorbed from the gastrointestinal (GI) tract following ingestion; inhalation exposure may be similar. Once absorbed, arsenic is stored in liver, kidneys, heart and lung while lower amount are present in muscle and neural tissues (8). In humans, absorbed inorganic pentavalent arsenic is bio-transformed to trivalent arsenic. Trivalent form of arsenic undergoes methylation to form less toxic compounds that are excreted in urine but some inorganic arsenic is excreted in the urine unchanged. Arsenic (V) is less toxic than arsenic (III). Arsenite (As III), the hydrated form of arsenic trioxide, is harmful as it is, owing to its facile covalent reaction with endogenous thiol groups especially, dithiols. Arsenic (III) is extensively bio-transformed into various methylated metabolites with markedly different toxic potential (9). Methylation of arsenic has long been regarded as a detoxification process because the pentavalent methylated arsenic metabolites; monomethylarsenic acid and dimethylarsinic acid are much less toxic and excreted more readily than As (III).

**Mechanism of arsenic Toxicity:** Arsenic toxicity is postulated to be primarily due to the binding of arsenic (III) to sulfhydryl group containing enzymes. Glutathione (GSH) plays a critical role in both the enzymatic and non-enzymatic reduction of pentavalent arsenicals to trivalent and in the complexation of arsenicals to form arsenicothiol during methylation process. The interaction of arsenic with glutathione and its related enzymes by changing their redox status and this may lead to the alterations of their biological function (10, 11). Recent studies have indicated that arsenic exerts toxicity by generating reactive oxygen species (ROS), but the mechanism is still unclear (12-14).

Trivalent intermediates of arsenic are involved in the formation of MMA and DMA (a pentavalent arsenic form) that might play an important role in arsenic toxicity as they are known to react with sulfhydryl groups and are highly toxic. Trivalent arsenic also inhibits pyruvate dehydrogenase (PDH), a multi sub-unit complex that needed lipoic acid as a cofactor for enzymatic activity. It has also been reported that MMA (III) is more potent inhibitor of PDH than arsenite (15). PDH oxidizes pyruvate to acetyl CoA, a precursor to intermediates of the citric acid cycle. The citric acid cycles degrade the intermediate, and this provides reducing equivalents to the electron transport system for ATP production. Inhibition of PDH may ultimately lead to decreased production of ATP. Inhibition of PDH may explain in part the depletion of carbohydrate observed in rats administered arsenic (15) (Fig. 1).

Oxidative stress is a relatively new theory of arsenic toxicity (16). Since about 1990, additional data supporting this theory and scientific acceptance of this mode of action have continued to occur. Dimethylarsine (a trivalent arsenic form) a metabolite of DMA produced by a process of reduction in vivo reacts with molecular oxygen forming (CH3)2As- radicals and superoxide anions. Exposure to these free radicals can lead to DNA damage (single strand breaks) (17). Oxidative stress theory for arsenic carcinogenicity can also be explained by its ability to cause cancer at high rates in the lung, bladder and skin. Human lung may be an organ responsive to arsenic carcinogenesis because of high partial pressure of oxygen and the fact that dimethylarsine, a gas is excreted via the lungs. In addition, human bladder may be
another organ responsive to arsenic carcinogenesis because of high concentration of DMA and MMA that is stored in the lumen of the bladder (Fig. 1).

**Signs and Symptoms of Arsenic Toxicity**

**Clinical Diagnosis of Arsenic Toxicity:** Clinical diagnosis for arsenicosis can be done by measuring blood, urine, and hair arsenic concentration. However, these diagnostics may be sensitive to duration of exposure for example blood arsenic concentration is only reliable within few days of acute exposure. In case of chronic exposure, urinary arsenic is the best indicator of current or recent body concentrations. Hair or fingernail arsenic concentrations may be useful in evaluating past exposure. Most investigators have used hair rather than nails arsenic because the former is easier to obtain in sufficient quantities. The diagnosis of chronic arsenic poisoning must also rely on the characteristic, clinical features of the typical skin lesions, debility, weight loss and neuropathy, since hair arsenic levels are supportive of the diagnosis and not self-sufficient to obtain complete clinical picture.

It is advisable that proper investigation should be carried out to define the various manifestations in chronic arsenosisis and these include routine hematological variable like haemoglobin, total and differential count, RBC morphology, urine and stool examination, chest X-ray, electrocardiogram determination of blood sugar, urea and creatinine. The chronic arsenosis produces protean manifestation which is evident from the report of the clinical features in 156 cases that had drinking arsenic contaminated water in West Bengal, in India. Further, although oxidative stress and other molecular biomarkers have been investigated to be used as diagnostic tools these are yet to be clinically used (18).

**Systemic Effects of Arsenic Toxicity:**

**Haem Synthesis Pathway:** In mammalian and avian tissues the principal product of haem synthesis pathway is haemferro-protoporphyrin IX, an essential component of various biological functions including oxygen transport systems, mixed function oxidative reactions and other oxidative metabolic processes. All eight steps of the haem synthesis are catalyzed by enzymes, which require functional sulfhydryl (-SH) group for optimal catalytic activity. Arsenic exposure has been known to influence the activity of several enzymes crucial to the haem synthesis pathway.

![Fig. 1. Mechanism of arsenic toxicity via different pathways](image-url)
enzymes of haem biosynthesis (19). It has been reported that arsenic exposure produces a decrease in ferrochelatase, and decrease in COPRO-OX and increase in hepatic 5-aminolevulinic acid synthetase activity (20). Sub-chronic exposure to arsenic has also been reported to inhibit ALA-S and ferrochelatase activities, which catalyze limiting steps in the heme synthesis pathway, leading to increases uroporphyrin (URO) and coproporphyrin and COPRO urinary excretion. Few recent studies also suggested a significant inhibition of blood d-aminolevulinic acid dehydratase (ALAD) after sub-chronic and chronic arsenic exposure (12).

Central Nervous System:
In children chronic exposure to arsenic, urine level of arsenic were inversely correlated with verbal IQ scores including verbal comprehension and long term memory (21). Previous reports confirmed that arsenic could cross blood brain barrier and produces alterations in whole rat brain biogenic amines levels in animals chronically exposed to arsenite (22). Many aspects of arsenic neurotoxicity remains to be investigated from its entrance into the brain, to the cellular and molecular targets that when, altered by arsenic exposure, lead to specific central nervous system dysfunctions.

Hepatotoxicity:
Arsenite is rapidly and extensively accumulated in the liver, where it inhibits NAD-linked oxidation of pyruvate or -ketoglutarate (23). This occurs by complexation of trivalent arsenic with vicinal thiols necessary for the oxidation of this substrate. Important feature of chronic arsenic toxicity in West Bengal is a form of hepatic fibrosis that causes portal hypertension, but does not progress to cirrhosis (24). Clinical examinations reveal liver to be swollen and tender. The analysis of blood revealed elevated levels of hepatic enzymes.

Dermal Toxicity:
Skin cancer has been associated with chronic inorganic arsenic exposure (25). Skin cancers are mostly monocentric but sometime multicentric cases are also found. Dermal changes most frequently reported in arsenic exposed humans include hyper pigmentation, melanosis, hyperkeratosis, warts, and skin cancer.

Carcinogenic Effects: One of the most severe adverse manifestations of chronic arsenic poisoning appears to be cancer (26). Numbers of epidemiological studies have reported a strong correlation between environmental, occupational and medical exposure of man to inorganic arsenic and cancer of skin and lungs. Epidemiological studies too have shown that chronic exposure to arsenic can result in an increased incidence of cancer of the lung, skin, bladder and liver (27-29). Arsenic-induced cancer has been extensively investigated and oxidative stress appears to be one of the most convincing mechanism underlying the etiology and progression of disease (30).

Developmental and reproductive toxicity: Chronic studies did not report any male reproductive organ pathology. However, in human studies a correlation has been observed between arsenic exposure and incidence of abortion. Higher spontaneous abortions and stillbirths were reported in the high arsenic area (arsenic in drinking water > 0.1 mg/L) compared to the control areas (31, 32).

Therapy
Chelation Therapy: Chelation is the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as ligand. Chelators act according to a general principle: the chelator form a complex with the respective (toxic) ion and these complexes reveal a lower toxicity and more easily eliminated from the body.

2, 3-dimercaptoprolo (BAL) is a traditional chelating agent that has been used clinically in arsenic poisoning since 1949 (33). Beside rapid mobilization of arsenic from the body, it causes a significant increase in brain arsenic. Other side effects include vomiting, headache, lachrymation, rhinorrhea and salivation, profuse sweating, intense pain in the chest and abdomen and anxiety. One of the chemical derivatives of
dimercaprol (BAL) is DMSA. DMSA is an orally active chelating agent, much less toxic than BAL and its therapeutic index is about 30 times higher. No significant loss of essential metals like zinc, iron, calcium or magnesium has been reported with DMSA administration. However in a double blind, randomized controlled trial study conducted on few selected patients from arsenic affected West Bengal (India) regions with oral administration of DMSA suggested that DMSA was not effective in producing any clinical and biochemical benefits or any histopathological improvements of skin lesions (34). Its distribution is predominantly extracellular; ultimately it is very well able to chelate arsenic from extracellular sites but does not able to chelate arsenic from intracellular sites. Thus, in lack of an arsenic chelator, researchers have been suggesting alternative therapeutic solutions for effective arsenic removal from body and clinical recovery. 

Combination Therapy: A new trend in chelation therapy has emerged recently, which is to use of combination therapy with more than one chelating agent instead of monotherapy (35-37). Vitamins, essential metals or amino acid supplementation during chelation therapy has also been found beneficial in increasing metal mobilization and providing recoveries in number of altered biochemical variables. Combined treatment with a chelating agent having antioxidant property and a thiol chelator could be a better treatment protocol for arsenic poisoning compared to monotherapy with a chelator (38, 39). Flora and his group have worked extensively in the field of arsenic therapy. They reported that co-administration of naturally occurring vitamins like vitamin E or vitamin C during administration of a thiol chelator like DMSA or MiADMSA may be more beneficial in the restoration of altered biochemical variables (particularly the effects on haem biosynthesis and oxidative injury) although it has only limited role in depleting arsenic burden. It was observed that optimum effects of chelation therapy could be achieved by combined administration of DMSA and MiADMSA (38, 40). It is evident from above that combination therapy is a new and a better approach to treat cases of metal poisoning (Fig. 2).

In search of an effective arsenic chelating agent, some mono and diesters of DMSA have been developed and tried against cases of...
experimental heavy metal poisoning. Monoisoamyl ester of DMSA (MiADMSA), a C₅ branched chain alkyl monoester of DMSA has found to be the most effective (41) against chronic arsenic toxicity. The metal chelators are given to increase the excretion of arsenic but unfortunately use of these chelators is comprised by number of drawbacks (42). These drawbacks open the search for new treatment which has no side effects and maximum clinical recovery in terms of altered biochemical variables because the total elimination of metals from the environment is not feasible.

Therapeutic supplementation: Chelation therapy forms mainstay for arsenic toxicity which must be developed and recommended as first line of therapy. However, for complete clinical recoveries various therapeutic supplementations also known as adjuvant have been suggested.

Nutritional intervention: Experimentally, excesses or deficiencies of essential trace elements and other dietary nutrients facilitate arsenic absorption. Selenium can also alleviate arsenic toxicity. Selenate partially prevents the uncoupling of oxidative phosphorylation by arsenate and decreases the teratogenic toxicity of arsenate in hamsters when both salts were injected simultaneously. Arsenic can also induce metallothionein (MT), a low molecular weight cysteine rich metal binding protein. This implies that arsenite can be detoxified by MT (43). Dietary antioxidants such as vitamin E and vitamin A may also be alleviating arsenic toxicity. Addition of vitamin E could at least in part prevent the arsenic-induced sever health manifestations (Fig. 2).

Role of Antioxidants: Oxidative stress has been popularly associated with metal toxicities and relevant diseased manifestations (44). Thus, employing antioxidants as therapeutic supplementations during metal chelation therapy serves beneficial effects. Antioxidants are substances, which inhibit or delay oxidation of a substrate while present in minute amounts. Nutritional antioxidants act through different mechanisms and in different compartments, but are mainly free radical scavengers: They directly neutralize free radicals, reduce the peroxide concentrations and repair oxidized membranes (39). They quench iron to decrease ROS production, via lipid metabolism, short-chain free fatty acids and cholesteryl esters neutralize ROS (45). Typical natural antioxidants include tocopherol, ascorbic acid, flavonoides, quercetin, carotene, cinnamic acid, peptides and phenolic compounds. Quercetin has been shown to scavenge superoxide radicals, protect from lipid peroxidation and chelate metal ions to form inert complexes (46).

Role of herbal Products: Plants parts like wood, bark, stem, leaf and pod may be important source of natural antioxidants. Aloe vera has been reported to possess antiulcer, antidiabetic, antioxidant and free radical scavenging activity (47). Centella asiatica improves learning and memory and possess antioxidant, antiulcer, and radioprotective activity. Thus, these herbal extracts when evaluated showed protection against arsenic-induced said manifestations (48-54). It is proven recently that shelled Moringa oleifera seed powder has ability to remove cadmium and arsenic from the aqueous system. Fourier transform infrared (FTIR) spectrometry highlights protein/amino acid – arsenic interactions responsible for sorption phenomenon of seed powder of Moringa oleifera (55-58). Garlic has been reported to prevent arsenic-induced oxidative stress and apoptosis and reversing altered clinical variables (59-61).

References
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Govinder J.S. Flora


