REVIEW ARTICLE

Metabolism and toxicity of arsenic: A human carcinogen

Pradosh Roy* and Anupama Saha

Department of Microbiology, Bose Institute, P-1/12, C.I.T. Scheme VII-M, Kolkata 700 054, India

Inorganic arsenic is considered the most potential human carcinogen, and humans are exposed to it from soil, water, air and food. In the process of arsenic metabolism, inorganic arsenic is methylated to monomethylarsonic acid and finally to dimethylarsinic acid, followed by excretion through urine. Thus, arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion, facilitating aberrant gene expression that results in carcinogenesis. Further, though arsenic is non-mutagenic, it interacts synergistically with genotoxic agents in the production of mutations, and also induces chromosome abnormalities and cell proliferation. Few epidemiological investigations in the arsenic endemic regions of West Bengal (India) have established that inorganic arsenicals have the potential to cause skin and lung cancers in humans. Studies on the genetic polymorphism in the arsenic methyltransferase(s) with the population exposed to arsenic, and characterization in the arsenic-induced mutational spectra may be useful for the development of molecular markers and therapeutics and for furthering the knowledge of arsenic-induced carcinogenesis.

IT has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential elements like As, Hg, Cd, and Pb[1]. In the second half of the nineteenth century, pollution of air, water, soil and food, especially due to these 'big four' metals has become a threat to the plant and animal communities, including the human race. Among these metals, arsenic exhibits a complex metabolism and is possibly the most abundant pollutant as well and a potential human carcinogen. Hence its origin and mobilization in the environment, biochemistry and bioavailability should be well understood to monitor our arsenic resources, since it has significant use in the present-day world.

*For correspondence. (e-mail: prodosh@boseinst.ernet.in)

Sources of different forms of arsenic: Human exposure and chronic arsenicism

Transformation and mobilization of arsenic in the environment

The principal natural reservoirs of arsenic are rocks. Release and mobilization of arsenic from these sources constitute the availability of this element in soil, water and air in various forms. As a result, arsenic is ubiquitous in our environment, and humans are always and unavoidably exposed to this toxic metalloid. Under normal ecological conditions, the level of arsenic bioavailability is not a threat for human health. Soils may contain arsenic levels between 0.1 and 40 ppm[2,3], if the underlying bedrock is not disturbed or redistributed by natural or pedogenic processes[2]. A large number of man-made arsenic compounds were used in agriculture as effective agents against pests, parasites or weeds and they gradually accumulated in the soil[2–4]. Further, concentrations of arsenic in the environment may be elevated due to certain other anthropological activities resulting in significant increase in the human exposure to arsenic[2–8] (Figure 1). The solubility, stability and cellular toxicity of various forms of arsenic are widely different. Thus, studies in the chemical form of arsenic especially the two inorganic arsenic species, arsenate (As^{5+}) and arsenite (As^{3+}), their transformation, persistence and bioavailability are pertinent in the understanding of levels of human exposure to arsenic.

Chemistry of inorganic arsenic in aquatic environment, especially with variable pH and oxygen availability, is unusually complex. The important feature is that in highly aerated condition arsenate salts are dissociated in all four arsenic acid (As^{5+}) species[9], H_3AsO_4, $H_2AsO_4^{1-}$, $HAsO_4^{2-}$ and AsO_4^{3-}. However, in mild reducing condition, arsenous (As^{3+}) acid species, H_3AsO_3, $H_2AsO_3^{1-}$ and $HAsO_3^{2-}$ may be stable. Arsenic acid (As^{5+}) is the least toxic of the inorganic forms and arsenous acid (As^{3+}) is more toxic *in vivo* than arsenic acid and also more inhibitory *in vitro*[10,11]. A large number of diverse chemical and biological reactions, viz. oxidation, reduction, adsorption, precipitation, methylation and volatilization participate actively in the cycling of this toxic element (Figure 2). These reactions control the availability of

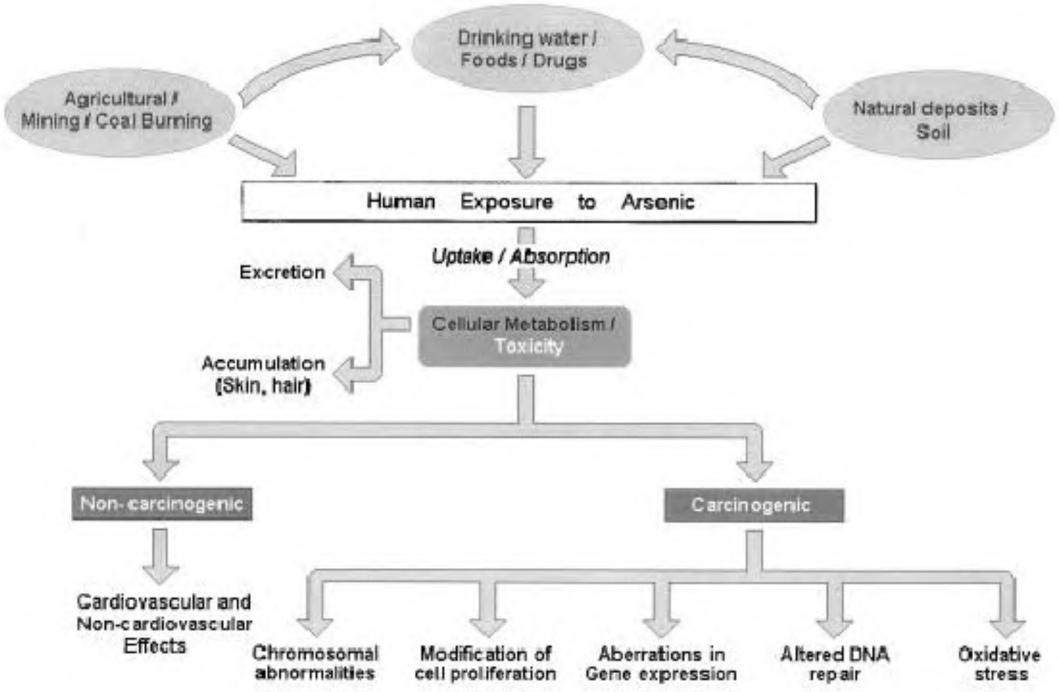

Figure 1. Sources of human exposure to arsenic and various modes of arsenic toxicity. Adapted from Azcue and Nriagu[4], Phillips[5] and Goering *et al.*[8].

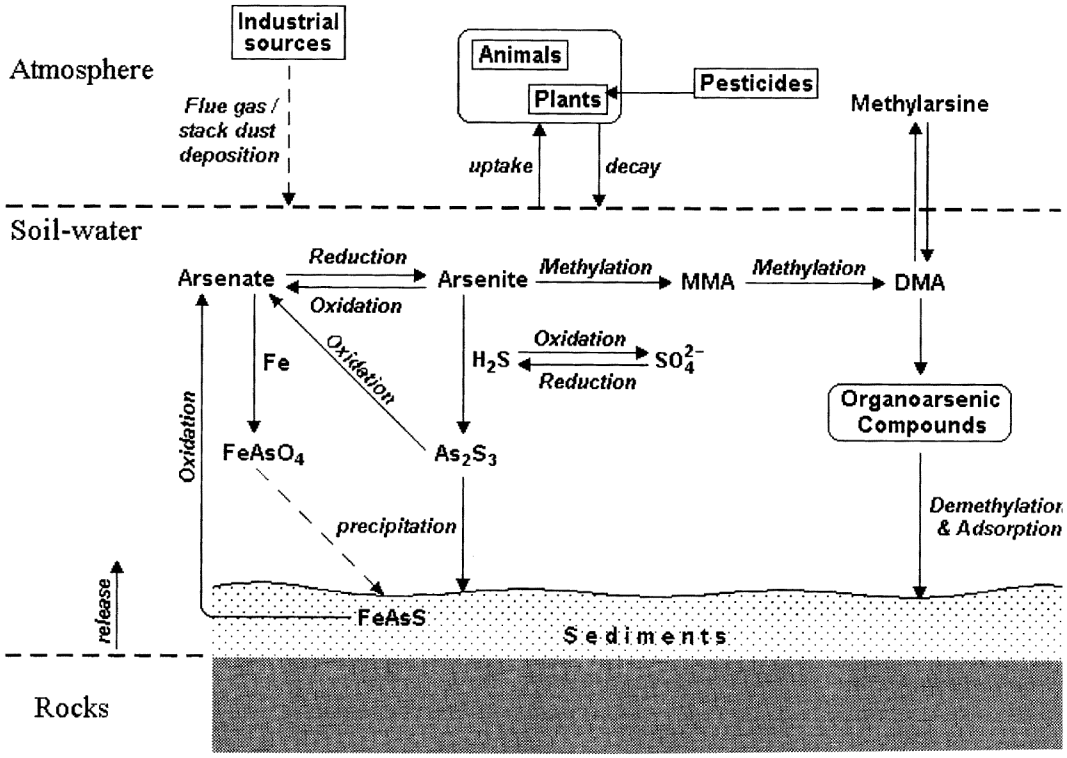

Figure 2. Arsenic cycle in the environment. Major reactions in the soil–water and sediment–rock systems to influence the environmental transport, distribution and availability of arsenic. Oxygen availability controls the arsenate–arsenite redox reactions. Adsorption and precipitation of arsenate and arsenite immobilize the soluble arsenic. Slow release of arsenic from rocks and sediments or oxidative dissolution of arsenopyrite (FeAsS) from sediments contribute flux of arsenic in the environment. Methylation of arsenite to monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) followed by other organoarsenic compounds, constitute the major biological reactions in the arsenic cycle. Adapted from Yan-Chu[2], Bhumbla and Keefer[7], Ferguson and Gavis[9], Knowles and Benson[11], Carter and Fairlamb[12].

arsenic, and hence, arsenic concentrations effectively exposed to humans are governed more by arsenic speciation than by the total amount of arsenic.

Arsenic poisoning or intoxication from therapeutic use

Arsenic is known and used since the history of human civilization due to its highly toxic properties. Until recently different arsenic compounds such as potassium arsenite, arsenic iodide or arsenic trichloride were used, as medication for a variety of illness[3,4]. Before the 'pre-antibiotic era', the discovery of salvarson (arsphenamine[3]) made it the main medicine used against syphilis. Generally uses of these drugs have been largely discontinued because of high toxicity of arsenic compounds. Nevertheless, several organic compounds or herbal products containing arsenic are still in use in human medicine. Tryparsamide ([4-[2-amino-2 oxoethyl]-amino]-phenyl] arsonic acid[3]) is used to treat African sleeping-sickness, a disease caused by parasitic protozoa of the *Trypanosoma brucei* subgroup[12]. Retinoic acid (RA) is a potent antileukaemic drug used in acute promyelocytic leukaemia (APL) patients[13]. Arsenic trioxide can also induce complete remission in APL patients[14]. However, arsenic trioxide triggers rapid degradation of PML-RA receptor protein chimera (a fusion product of PML and RA receptor protein gene[15]) from both RA-sensitive and RA-resistant APL patients[16]. Understanding the molecular basis of differences in the effects of arsenic trioxide and RA may guide the clinical use of arsenic compounds and could be effectively introduced into the management of leukaemia that does not respond to RA. In fact, arsenite was shown to induce apoptosis in different leukaemia cell lines[17]. Thus, arsenic is used in the Ayurvedic system of medicine in India to control blood counts of patients with haematological malignancies[18]. In a routine screening test made by the competent authority of the National Fish and Wildlife Forensics Laboratory in Ashland (USA), different types of medicinal herbal balls made in China were found to contain potentially dangerous amounts of arsenic[19]. The observation that, exposure to Chinese proprietary medicines containing inorganic arsenic induced cutaneous and systemic malignancies having long latency periods for the development of arsenical keratoses or squamous cell carcinoma, strongly recommended that surveillance programmes must be introduced to restrict the sale of such medicines that may contain inorganic arsenic[20]. Similarly arsenic was found in notable quantities in two homeopathic medicines, though, no warnings of possible dangers from ingestion appeared on the labels[21]. A 42-year-old man had developed arsenical keratoses ten years after a two-year treatment with Fowler's solution (1-% solution of arsenic trioxide)[22]. It is apparent that the side effects or toxicity of arsenic had not been adequately considered, rather they were ignored by the practitioners and users.

Exposure due to industrial activities

Arsenic present in various metal ores or coal is released during the smelting process or in coal-burning, which produces stack dust and flue gas to contaminate the soil and water with arsenic, downwind from the operation[7] (Figure 2). As a result arsenic pollution in mining places and in smelting or coal-burning in thermal power plants continues to be a severe health problem. The environmental survey with the epidemiological study, conducted between 1987 and 1994 in the southern part of Thailand where mining was the primary resource, recorded prevalence of chronic arsenic poisoning in the population of the affected districts of Thailand[23]. In Kolkata, a factory that was manufacturing the pesticide, Paris green (K-acetoarsenite), contaminated the subsurface and even the underground aquifer surrounding the factory[24,25], affecting several thousand people of the region. A clinical investigation[24] of 20 patients from this place revealed significant prevalence of keratosis, liver damage and respiratory disease. The study also noted that metabolism of arsenic in the body after its ingestion, and its clearance were quite variable in man and no effective treatment protocol was available for the amelioration of chronic arsenic toxicity. Similarly, in the vicinity of a lead factory at Kolkata, the soil and surface water were found highly contaminated not only with lead but also with several other toxic metals, including arsenic[26]. All these reports suggest that effective environmental safety measures should be innovated by the industry.

Arsenic pollution in drinking water

The arsenic calamity reported from many parts of the world[27–33] is thought to be due to contamination of underground water and of geogenic origin. The first two epidemiological studies of arsenic-induced dermatoses from consuming arsenic-contaminated water were conducted by Tseng[27] and Saha[28] in Taiwan and West Bengal (India), respectively. Subsequently, Saha[29] made an extensive survey during the period of 1983–1987 from 61 villages of 7 districts of West Bengal (WB). He had detected 1214 cases of chronic arsenical dermatoses (melanosis and keratosis), having skin cancer in 6 cases. Saha also noted that all the affected individuals were villagers of poor economic status (mostly agricultural labourers) who were exposed to low doses of arsenic for several years. This report was further substantiated by other workers[31,32] indicating that about

two lakh people (more than 0.6% of total population) in the arsenic-endemic regions of WB have arsenical skin lesions, and one million people are at risk due to consumption of water having arsenic concentrations 10 to 20-fold higher than the maximum permissible limit of 0.05 ppm[3].

The source of arsenic resulting in pollution of aquifer in this vast region of WB is thought to be geogenic. It is suspected that arsenopyrite-rich sediment is being slowly solubilized, because of increased oxygen availability due to the extensive removal of groundwater through shallow or deep tube-well used for irrigation[34–36]. From the hydrogeological point of view, a younger deltaic deposit brought by the river Ganga and sedimented at the right side of the river, is the source of arsenic in this region that spans a vast area of WB and the neighbouring country of Bangladesh[36]. In a recent survey report, conducted jointly by School of Environmental Studies, (Kolkata) and Dhaka Community Hospital, Dhaka (Bangladesh), the investigators claimed that arsenic calamity in Bangladesh is more severe than in WB and fifty million people within thirty-four districts of Bangladesh would be at risk, in the absence of a proper 'watershed management'[36].

Control actions for the prevention of arsenic contamination in drinking water

Several methods such as coagulation and precipitation by ferric chloride or alum, or adsorption onto activated carbon and alumina, or use of ferric hydroxide-impregnated adsorbents are effective for the partial removal of soluble arsenic from water[37]. However, it is not feasible to implement such a chemical treatment or process *in situ* to stop arsenic contamination of underground aquifer. A natural process of mitigation of aquifer was recommended by recharging rainwater to the shallow aquifer and simultaneously restricting the future tube-wells to a depth of 50 m (ref. 36). But the actual origin and mobilization of arsenic in the affected aquifer should be identified; otherwise, any control actions in such a devastating environmental problem may lead to an uncontrollable situation. Recently, the All India Institute of Hygiene and Public Health has developed and installed several hand pump-attached arsenic removal plants (ARPs), made on the principle of oxidation–coagulation–flocculation–sedimentation–filtration, in arsenic-affected villages of WB, to provide arsenic-free drinking water[34]. The cost, feasibility and maintenance of the ARPs, and disposal of the toxic residue of arsenic (which would be adsorbed in an ARP) in particular, remain to be considered in view of the efficiency of the village people, who will be the actual beneficiaries and responsible in the viability of the process.

Metabolism of arsenic

Arsenic has no known nutritional requirement to the biological world, yet the metabolic process of this element in the biological species, including humans has been evolved. It is likely that metabolism of arsenic, like other toxic metals, is associated with the conversion of the most potentially toxic forms of this element to the less toxic form, followed by accumulation in or excretion from the cell. Although many toxic metals such as mercury or cadmium are biomagnified through the food chain, arsenic does not appear to be biomagnified[9,38].

Biomethylation – The general principle of arsenic detoxification

Biomethylation of arsenic is considered the primary detoxification mechanism, since the highly reactive species of inorganic arsenic are potentially more toxic to the living world, including humans. A large number of diverse species of yeast, fungi, algae, plants and animals were found to transform inorganic arsenic compounds to the methyl derivatives[38–41]. The organo-arsenic species, mostly of methyl derivatives such as arsenosugars, arsenobetaine, arsenocholine and arseno-lipids[11], are widespread in aquatic organisms, including shrimps, lobsters, fish, sea weeds, and in many species of marine animals[42,43]. It is unlikely that consumption of organoarsenic compounds in those organisms constitutes a hazard from arsenic poisoning[9]. However, ingestion of certain seafood was shown to elevate the urinary arsenic content beyond that expected for the current maximum contaminant level[44].

In higher organisms, inorganic arsenic is methylated to monomethylarsonic acid (MMA) and finally to dimethyl-arsinic acid (DMA) by the methyl donor, S-adenosyl-methionine (SAM)[45], catalysed by methyltransferases in the presence of glutathione[41]. These methyl derivatives are thousand-fold less potent as mutagenic agents than the inorganic arsenicals, as evident from a mouse lymphoma assay[46]. A methylation threshold hypothesis has been proposed[47], stating that after exposure to inorganic arsenic, methylation capacity begins to decline after a certain level, thus increasing the toxic effects of inorganic arsenic. The trivalent arsenicals are preferred substrates for methylation reactions and hence the reduction of arsenic from pentavalent to trivalent may be a critical step in the control of the rate of metabolism of arsenic[48]. The evidence that DMA excretion culminates after a few days, when the excretion of the inorganic form is substantially increased (while that of MMA is still elevated), seems to confirm the existence of two successive methylating-enzyme activities. Arsenic is converted in the liver to the less toxic methylated form that is excreted via the urine and follows a triphasic model with periods of 28 h, 59 h

and 9 days, respectively with the half-life ranging between 27 and 86 h, of different arsenic species showing the following gradient As^{5+} < MMA < As^{3+} < DMA (ref. 49). The DNA methyl transferases require SAM and as a result arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion. On the other hand, DNA hypomethylation occurs concurrently with malignant transformation and in the presence of depressed levels of SAM. Thus arsenic-induced hypomethylation facilitates aberrant gene expression that results in carcinogenesis[8,50].

To evaluate the methylation of arsenic as the detoxification mechanism in mammals, it is necessary to show the association between methyltransferase activity and toxicity of this metalloid. This issue was addressed in a recent symposium at the 37th Annual Meeting of the Society of Toxicology held at Seattle, USA[8]. The results presented and summarized in the area of arsenite methyltransferase showed striking diversity in this enzyme among the tested animals. The new world monkey (Marmoset *Callithrix jacchus*), chimpanzee and guinea pigs are deficient in arsenic methyltransferase, while rhesus monkey, rabbit and hamster have both arsenite and MMA methyltransferases[51–53]. An obvious question arises regarding the consideration of methylation as the primary detoxification process, since a few of the mammals are deficient in the primary enzyme of this metabolic process. With humans having been exposed to high levels of arsenic in drinking water, urinary MMA levels were found to increase several fold during treatment with sodium 2,3-dimercapto-1-propane sulfonate [DMPS (Dimaval^{R}), a chelating agent used to treat metal intoxication][54]. It is apparent that chronic arsenic exposure had influenced adversely the methylation of MMA to DMA, though the actual physiological cause of reduced rate of methylation due to arsenic exposure is yet unclear. Arsenite, having a high affinity for thiol groups in proteins, can form complexes with vicinal thiols[14] and inhibit more than 200 enzymes[51]. So, inhibition of methyltransferase by arsenite could affect arsenic metabolism. On the other hand, genetic polymorphism in the arsenite MMA methyltransferases might contribute to the observed aberrations in arsenic metabolism and variations in degree of susceptibility of arsenic exposure within a population or of different geographic regions[52,55]. So, studies in the genetic polymorphism, using amino acid sequence [or the coding gene] of arsenite methyl tranferases as a probe[40] in the arsenic-endemic large population of WB should provide insight into the genetic basis of arsenic metabolism.

Toxicity aspects of inorganic arsenic

The various factors which can affect the toxicity of arsenic are difficult to investigate, because of its ability to convert between oxidation states and organometalloidal forms[56]. It can participate in oxidation–reduction reactions with species of oxygen like O_2, O_2^- and H_2O_2 and hence these reactions may be modulated by endogenous reducing agents such as glutathione, ascorbate and tocopherol[57]. The reaction also depends on the pH and/or the presence of organic ligands that form complexes with the metal or metalloid. More importantly, complexity in the investigations of arsenic toxicity is obvious because of the competition or interference of arsenic with normal metabolic pathways. Practically very little information is available in the arsenic uptake and/or efflux system. Multidrug resistance-associated protein (MRP) is an ATP-coupled transport pump[58] that extrudes glutathione-conjugated arsenite from lung cancer-cells, indicating that the MRP is functionally similar to ATP-coupled arsenite efflux pump that confers arsenite resistance[59] to bacterial cells. Hence, studies on the molecular biology of the arsenic resistance in microbes might be useful in the investigations of arsenic transport system in higher organisms, including humans.

The acute effects due to consumption of arsenic at a high dose are different from chronic effects due to a long-time exposure of low doses of arsenic. The influence of gender, age, health-status parameters, nutrition and various lifestyle factors on chronic arsenic-toxicity was investigated[60–62]. These investigations had revealed that urinary arsenic levels were higher for men than for women, and increased with age up to 60 years and then decreased. The epidemiological studies in Thailand[23], Taiwan[27,61], West Bengal[29] and Bangladesh[32] revealed that undernourishment and deficiency in protein diet[8,62] in particular, were significantly associated with increased prevalence of arsenic carcinogenesis. In exposed human populations, duration of consumption of high-arsenic water, average and cumulative arsenic exposure also showed a dose–response relationship with tumours of skin, lung, bladder and liver.

Cytotoxicity, morphological neoplastic transformation and cellular uptake were determined, to compare the effect between trivalent and pentavalent arsenic[60,63]. Cytotoxicity of arsenite (As^{3+}) was found to be greater than that of arsenate (As^{5+})[64]. Arsenic species was also reported to increase in GSH levels in cultured Chinese hamster V79 cells and was associated with changes in gamma-glutamyl-cysteine synthetase activity, cysteine uptake and utilization of cysteine[65]. Arsenate accelerates the rate of ITP (inosine triphosphate) hydrolysis and inhibits both Ca^{2+} and Sr^{2+} uptake[66]. This perturbation of intracellular Ca^{2+} homeostasis activates protein kinase C (PKC) activity, which may play an important role in arsenite-induced genotoxicity[67]. Data obtained with women and children exposed to high levels of arsenic through drinking water showed genotoxic effects in peripheral blood lymphocytes and micronuclei in binucleated cells that seemed to be originated from whole chromosome loss[68]. A similar finding of arsenite-induced micronuclei was reported with X-ray hypersensitive Chinese

hamster ovary cells[69]. Further, in human fibroblasts, arsenite induces chromosome ends-reduplication and inhibits ser/thr protein phosphatase activity and enhances phosphorylation levels of a small heat-shock protein, hsp27 (ref. 70).

Arsenic is a pro-oxidant and thus may cause lipid peroxidation[71], protein and enzyme oxidation and GSH depletion, DNA oxidation and DNA adducts[72]. Further, arsenic generates reactive oxygen species[55,69,73] like nitric oxide; reactive oxygen species are known to induce poly ADP-ribosylation which is implicated in DNA repair, signal transduction and apoptosis. As a result, arsenite may induce DNA strand-breaks and NAD depletion[72]. Hence the genotoxic effects of arsenic compounds may be connected with an inhibition of DNA repair or the induction of oxidative stress[74]. In fact, metabolic methylation of inorganic arsenic to DMA is involved in induction of DNA damage and DNA single-strand breaks resulting from the inhibition of repair polymerization[50,75,76] and hence is a genotoxic-enhancing process. It is thus likely that arsenic-mediated DNA–protein interactions may play a major role in arsenic carcinogenesis and the induced protein associated DNA-strand breaks could provide an explanation for chromosome aberration[77].

Treatment of arsenic toxicity

Knowledge of the physiological processes of arsenic metabolism and the biochemical pathways involved in it coupled with the knowledge of arsenic chemistry can be used to device treatments of arsenic toxicity. Glutathione peroxidase and catalase modulate the genotoxicity of arsenite, but the former is more effective than catalase in defending against arsenite toxicity, indicating that increasing the intracellular antioxidant level may have preventive or therapeutic effects in arsenic poisoning[66]. Chelation therapy with dimercaprol (BAL)[29], dimaval^{R} (DMPS) or meso-2,3-dimercaptosuccinate acid (DMSA) is effective[8]. In case of severe intoxication by arsenicals, when the point of no return is a limiting factor, BAL is the only arsenic-antidote[78]. In a long-term investigation and treatment of chronic arsenical dermatoses with BAL, Saha[29] estimated arsenic concentration in urine of patients before, during and on completion of the course of BAL injections. He reported that arsenic in urine started appearing from the second or the third day of therapy and continued up to the twelfth or twentieth day of injection, according to the severity of the disease. In a recent study in Chile[54], with the population exposed to arsenic through drinking water, MMA level was found to increase by about 30% in the total urine arsenic after DMPS challenge accompanied by a substantial decrease in DMA. Although treatment with those chelating agents is effective in reducing arsenic burden, the altered biochemical variables responded less favorably to chelation therapy[79].

Arsenic – A human carcinogen

Arsenic toxicity and its potentiality in the development of cancer were known for a very long time, but carcinogenicity could not be directly tested because arsenic is yet to be shown to cause cancer in rodents. The reasons for these apparent interspecies differences are not known but may be related to distinct detoxification mechanisms or capabilities between humans and rodents[46]. Consequently, the precise cellular mechanism of arsenic-induced carcinogenesis (arsenicosis) is not yet uncovered, though, in a large number of recent publications and in several international conferences held in different parts of the world, we find widespread interest regarding arsenic exposure and human health.

Molecular mechanism of arsenic-induced carcinogenesis

The mechanism of action of inorganic arsenic leading to cancer, remains elusive. There is no evidence that inorganic arsenic species react covalently with DNA-like organic carcinogens. But, to be a carcinogen it has to act in some way to alter the regulation of cell replication.

In the study with human keratinocyte cultures, arsenite was shown to increase cell proliferation via the production of keratinocyte-derived growth factors; analysis of gene expression in samples of skin lesions obtained from patients exposed to arsenic via drinking water also showed similar alterations in growth factor expression[80]. In another investigation with arsenic-related skin neoplasm and arsenic keratosis, 8-hydroxy-2′-deoxyguanosine level was found to be significantly higher than the control samples[81]. The authors suggested that reactive oxygen species caused the DNA-base modification and thus was associated with arsenic-induced human skin cancer. Few recent reports on arsenic-induced DNA methylation have reinforced its carcinogenic potential. Both hypo- and hyper-methylation of DNA could cause aberrant expression of genes (such as oncogenes or tumour-suppressor genes) which in turn cause abnormality in cell proliferation leading to carcinogenesis. It was shown that in human lung adenocarcinoma A549 cells, promoter region of tumour suppressor gene p53 was methylated when exposed to arsenite[82]. This was further evident from the observation that, expression of tumour suppressor gene p53 in different cell lines was increased with arsenic and mutation in p53 was shown to increase sensitivity to arsenic cytotoxicity[83]. Arsenite was also shown to alter nuclear binding levels of transcription factors AP-1, NF-kappaB, Sp1 and YB-1 to their respective *cis*-acting elements in human breast and rat hepatoma cells[84]. The results implicate that arsenite affects specific signalling pathways within cells, to selectively modulate gene expression.

Conclusions

Arsenic, a metalloid, ranked first in a list of 20 hazardous substances by the Agency for Toxic Substances and Disease Registry and United States Environmental Protection Agency[8]. The evidence for carcinogenicity of arsenic is very strong in humans, but weak in animals, a unique and different scenario than is found for other carcinogens[86]. At present there are no recognized models for the study of arsenic-induced carcinogenesis. It is non-mutagenic in bacterial or human cells, though it interacts synergistically with genotoxic agents in the production of mutations[72,85,86]. However, it is convincingly established that arsenicosis is mediated through chromosome abnormalities, modification of gene expression, and cell proliferation due to oxidative stress and other uncharacterized or poorly defined physiological modifications or aberrations. Further, studies on the development of an animal model, characterization of the effect of arsenic on DNA repair and mutational spectra, and searching the genetic polymorphism in the arsenic methyltransferase(s) with the population exposed to arsenic may be useful to find out suitable molecular markers and therapeutics, and for the advancement of the knowledge of susceptibility to arsenic-induced carcinogenesis in the human population[72].

Exposures to arsenic large enough to cause acute toxic effects would be easily recognized and the source of exposure would be found and eliminated. But the problem lies in the fact that low doses of arsenic that would be too low to cause overt acute toxicity, finally be recognized after a long time with the development of cancer. This has demanded a serious effort to trace all the possible sources that cause human exposure of this 'king of poison'[4]. Finally, it is worth mentioning that arsenic poisoning in humans should not be considered a natural phenomenon, rather it is due to wrong policy of uncontrolled industrialization and ignorance to develop an effective water management of surface-water resources.

1. Clarkson, T., *Environ. Health Perspect.*, 1995, **103**, 9–12.
2. Yan-Chu, H., in *Arsenic in Environment. Part I: Cycling and Characterization* (ed. Nriagu, J. O.), John Wiley & Sons Inc., 1994, pp. 17–49.
3. Report of World Health Organization, Geneva, published by joint sponsorship under the United Nations Environmental Programme, International Labour Organization, and World Health Organization, Geneva, Switzerland, 1981, vol. 18.
4. Azcue, J. M. and Nriagu, J. O., in *Arsenic in Environment. Part I: Cycling and Characterization* (ed. Nriagu, J. O.), John Wiley & Sons Inc., 1994, pp. 1–15.
5. Phillips, D. J. H., *ibid*, pp. 263–268.
6. Tamaki, S. and Frankenberger, Jr., *Rev. Environ. Contam. Toxicol.*, 1992, **124**, 79–110.
7. Bhumbla, D. K. and Keefer, R. F., in *Arsenic in Environment. Part I: Cycling and Characterization* (ed. Nriagu, J. O.), John Wiley & Sons Inc., 1994, pp. 51–82.
8. Goering, P. L., Aposhian, H. V., Mass, M. J., Cebrian, M., Beck, B. D. and Waalkes, M. P., *Toxicol. Sci.*, 1999, **49**, 5–14.
9. Ferguson, J. F. and Gavis, J., *Water Res.*, 1972, **6**, 1259–1274.
10. Cervantes, C., Ji, G., Ramirez, J. L. and Silver, S., *FEMS Microbiol. Rev.*, 1994, **15**, 355–367.
11. Knowles, F. C. and Benson, A. A., *Trends Biochem. Sci.*, 1983, **8**, 178–180.
12. Carter, N. S. and Fairlamb, A. H., *Nature*, 1993, **361**, 173–176.
13. Castaigne, S., Chomienne, C., Daniel, M., Ballerini, P., Berger, R., Fenaux, P. and Degos, L., *Blood*, 1990, **76**, 1704–1709.
14. Muller, S., Matunis, M. J. and Dejean, A., *EMBO J.*, 1998, **17**, 61–70.
15. Kastner, P. *et al.*, *EMBO J.*, 1992, **11**, 629–642.
16. Shao, W. *et al.*, *J. Natl. Cancer Inst.*, 1998, **90**,124–133.
17. Lu, M., Levin, J., Sulpice, E., Sequeira-Le, G. A., Alemany, M., Caen, J. P. and Han, Z. C., *Exp. Hematol.*, 1999, **27**, 845–852.
18. Ireleaven, J., Meller, S., Farmer, P., Birchall, D., Goldman, J. and Piller, G., *Leuk. Lymphoma*, 1993, **10**, 343–345.
19. Holmes, B., *New Sci.*, 1995, 5.
20. Wong, S. S., Tan, K. C. and Goh, C. L., *J. Am. Acad. Dermatol.*, 1998, **38**, 179–185.
21. Kerr, H. D. and Saryan, L. A., *J. Toxicol. Clin. Toxicol.*, 1986, **24**, 451–459.
22. Saas, O., Grosshans, E. and Simonart, J. M., *Dermatology*, 1993, **186**, 303–305.
23. Choprapawan, C. and Rodcline, A., in *Arsenic: Exposure and Health Effects* (eds Abernathy, C. O., Calderon, R. L. and Chappel, W. R.), Chapman & Hall UK, 1997, pp. 69–77.
24. Guha Mazumder, D. N. *et al.*, *ibid*, pp. 113–123.
25. Samanta, G. *et al.*, *Curr. Sci.*, 1998, **75**, 123–137.
26. Chatterjee, A. and Banerjee, R. N., *Sci. Total Environ.*, 1999, **9**, 175–185.
27. Tseng, W. P., *Environ. Health Perspect.*, 1977, **19**, 109–119.
28. Saha, K. C., *Indian J. Dermatol.*, 1984, **29**, 37–46.
29. Saha, K. C., *Indian J. Dermatol.*, 1995, **40**, 1–12.
30. Narang, A. P., *J. Toxicol. Clin. Toxicol.*, 1987, **25**, 287–295.
31. Mandal, B. K. *et al.*, *Curr. Sci.*, 1996, **70**, 976–986.
32. Dhar, R. K. *et al.*, *Curr. Sci.*, 1997, **73**, 48–59.
33. Guo, H.-R., in *Arsenic: Exposure and Health Effects* (eds Abernathy, C. O., Calderon, R. L. and Chappel, W. R.), Chapman & Hall, UK, 1997, pp. 243–259.
34. Newsletter of Indian Training Network and All India Institute of Hygiene and Public Health, Kolkata, 1996, vol. 3, pp. 1–12.
35. Bagla, B. and Kaiser, J., *Science*, 1996, **274**, 174 –175.
36. Mallick, S. and Rajagopal, N. R., *Curr. Sci.*, 1996, **70**, 956–958.
37. Hlavay, J. and Polyak, K., in *Arsenic: Exposure and Health Effects* (eds Abernathy, C. O., Calderon, R. L. and Chappel, W. R.), Chapman & Hall, UK, 1997, pp. 382–392.
38. Maeda, S., in *Arsenic in Environment. Part I: Cycling and Characterization* (ed. Nriagu, J. O.), John Wiley & Sons Inc., 1994, pp. 155–187.
39. Cullen, W. R. and Reimer, K. J., *Chem. Rev.*, 1989, **89**, 713–764.
40. Aposhian, H. V., *Annu. Rev. Pharmacol. Toxicol.*, 1997, **37**, 397–419.
41. Styblo, M. and Thomas, D. J., in *Arsenic: Exposure and Health Effects* (eds Abernathy, C. O., Calderon, R. L. and Chappel, W. R.), Chapman & Hall, UK, 1997, pp. 283–295.
42. Edmonds, J. S. and Francesconi, K. A., *Nature*, 1981, **289**, 602–604.
43. Edmonds, J. S. and Francesconi, K. A., *Experimentia*, 1987, **43**, 553–557.
44. Buchet, J. P., Pauwels, J. and Lauwerys, R., *Environ. Res.*, 1994, **66**, 44–51.
45. Buchet, J. P. and Lauwerys, R., *Arch. Toxicol.*, 1985, **57**, 125–129.
46. Moore, M. M., Harrington-Brock, K. and Doerr, C. L., *Mutat. Res.*, 1997, **386**, 279–290.

47. Hopenhayn-Rich, C., Smith, A. H. and Goeden, H. M., *Environ. Res.*, 1993, **60**, 161–177.
48. Styblo, M., Yamauchi, H. and Thomas, D. J., *Toxicol. Appl. Pharmacol.*, 1995, **135**, 172–178.
49. Apostoli, P., Alessio, L., Romeo, L., Buchet, J. P. and Leone, R., *J. Toxicol. Environ. Health*, 1997, **52**, 331–342.
50. Zhao, C. Q., Young, M. R., Diwan, B. A., Coogan, T. P. and Waalkes, M. P., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10907–10912.
51. Abernathy, C. O. *et al.*, *Environ. Health Perspect.*, 1999, **107**, 593–597.
52. Healy, S. M., Zakharyan, R. A. and Aposhian, H. V., *Mutat. Res.*, 1997, **386**, 229–239.
53. Vehter, M., Couch, R., Nermell, B. and Nilsson, R., *Toxicol. Appl. Pharmacol.*, 1995, **133**, 282–288.
54. Aposhian, H. V. *et al.*, *J. Pharmacol. Exp. Ther.*, 1997, **282**, 192–200.
55. Vehter, M., Concha, G., Nermell, B., Nilsson, R., Dulout, F. and Natarajan, A. T., *Eur. J. Pharmacol.*, 1996, **293**, 455–462.
56. Thompson, D. J., *Chem. Biol. Interact.*, 1993, **88**, 89–114.
57. Carter, D. E., *Environ. Health Perspect.*, 1995, **103**, 17–19.
58. Dey, S., Dou, D. and Rosen, B. P., *J. Biol. Chem.*, 1994, **269**, 25442–25446.
59. Dey, S., Ouellette, M., Lightbody, J., Papadopoulou, B. and Rosen, B. P., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 2192–2197.
60. Gonsebatt, M. E. *et al.*, *Mutat. Res.*, 1997, **386**, 219–228.
61. Hsueh, Y. M., Chen, G. S., Wu, M. M., Yu, H. S., Kuo, T. L. and Chen, C. J., *Br. J. Cancer*, 1995, **71**, 109–114.
62. Kristiansen, J., Christensen, J. M., Iversen, B. S. and Sabbioni, E., *Sci. Total Environ.*, 1997, **204**, 147–160.
63. Bertolero, F., Pozzi, Z., Sabbioni, E. and Saffiotti, U., *Carcinogenesis*, 1987, **8**, 803–808.
64. Jacobson-Kram, D. and Montalbano, D., *Environ. Mutagen.*, 1985, **7**, 787–804.
65. Ochi, T., *Arch. Toxicol.*, 1995, **7**, 730–740.
66. Alves, E. W. and de Meis, L., *Eur. J. Biochem.*, 1987, **166**, 647–665.
67. Liu, Y. C. and Huang, H., *J. Cell. Biochem.*, 1997, **64**, 423–433.
68. Dulout, F. N. *et al.*, *Mutat. Res.*, 1996, **370**, 151–158.
69. Wang, T. S., Shu, Y. F., Liu, Y. C., Jan, K. Y. and Huang, H., *Toxicology*, 1997, **121**, 229–237.
70. Huang, R. N., Ho, I. C., Yih, L. H. and Lee, T. C., *Environ. Mol. Mutagen.*, 1995, **25**, 188–196.
71. Schlenk, D., Wolford, L., Chelius, Steevens, J. and Chan, K. M., *Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.*, 1997, **118**, 177–183.
72. Lynn, S., Lai, H. T., Gurr, J. R. and Jan, K. Y., *Mutagenesis*, 1997, **12**, 353–358.
73. Lynn, S., Shiung, J. N., Gurr, J. R. and Jan, K. Y., *Free Radical Biol. Med.*, 1998, **24**, 442–449.
74. Gebel, T., *Chem. Biol. Interact.*, 1997, **107**, 131–144.
75. Yamanaka, K., Hayashi, H., Tachikawa, M., Kato, K., Hasegawa, A., Oku, N. and Okada, S., *Mutat. Res.*, 1997, **394**, 95–101.
76. Hartwig, A., Groblinghoff, U. D., Beyersmann, D., Natarajan, A. T., Filon, R. and Mullenders, L. H., *Carcinogenesis*, 1997, **18**, 399–405.
77. Uong, J. I. and Luo, X. M., *Mutat. Res.*, 1993, **302**, 97–102.
78. Muckter, H., Liebl, B., Reichi, F. X., Hunder, G., Walther, U. and Fichtl, B., *Hum. Exp. Toxicol.*, 1997, **16**, 460–465.
79. Tripathi, N., Kanan, G. M., Pant, B. P., Jaiswal, D. K., Malhotra, P. R. and Flora, S. J., *Toxicol. Lett.*, 1997, **92**, 201–208.
80. Germoolec, D. R. *et al.*, *Am. J. Pathol.*, 1998, **153**, 1775–1785.
81. Matsui, M. *et al.*, *J. Invest. Dermatol.*, 1999, **113**, 26–31.
82. Mass, M. J. and Wang, L., *Mutat. Res.*, 1997, **386**, 277.
83. Salazar, A. M., Ostrosky, W. P., Menendez, D., Miranda, E., Garcia, C. A. and Rojas, E., *Mutat. Res.*, 1997, **381**, 259–265.
84. Kaltreider, R. C., Pesce, C. A., Ihnat, M. A., Lariviere, J. P. and Hamilton, G. W., *Mol. Carcinogen.*, 1999, **25**, 219–229.
85. Wiencke, J. K., Yager, J. W., Varkonyi, A., Hultner, M. and Lutze, L. H., *Mutat. Res.*, 1997, **386**, 335–344.
86. North, D. W., Gibb, H. J. and Abernathy, C. O., in *Arsenic: Exposure and Health Effects* (eds Abernathy, C. O., Calderon, R. L. and Chappel, W. R.), Chapman & Hall, UK, 1997, pp. 406–423.

ACKNOWLEDGEMENTS. The work was supported in part by the Department of Biotechnology, Government of India, under the project BT/PR0578/PID/11/34/97. Debashis Majumder provided expertise in the preparation of figures. The authors are grateful to Paramita Basu and Ajoy K. Halder, Department of Health, West Bengal for providing valuable information and literature. A.S. is thankful to Council of Scientific and Industrial Research for award of a research fellowship.

Received 26 June 2001; revised accepted 10 October 2001