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Effects of individual and combined exposure to sodium arsenite and sodium fluoride on tissue oxidative stress, arsenic and fluoride levels in male mice

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Abstract

Arsenic and fluoride are potent toxicants, widely distributed through drinking water and food and often result in adverse health effects. The present study examined the effects of sodium *meta*-arsenite (100 mg/l in drinking water) and sodium fluoride (5 mg/kg, oral, once daily), administered either alone or in combination for 8 weeks, on various biochemical variables indicative of tissue oxidative stress and cell injury in Swiss albino male mice. A separate group was first exposed to arsenic for 4 weeks followed by 4 weeks of fluoride exposure. Exposure to arsenic or fluoride led to a significant depletion of blood δ-aminolevulinic acid dehydratase (ALAD) activity and glutathione (GSH) level. These changes were accompanied by increased level of blood and tissues reactive oxygen species (ROS) level. An increase in the level of liver and kidney thiobarbituric acid reactive substance (TBARS) along with a concomitant decrease in the activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) and reduced GSH content were observed in both arsenic and fluoride administered mice. The changes were significantly more pronounced in arsenic exposed animals than in fluoride. It was interesting to observe that during combined exposure the toxic effects were less pronounced compared to the effects of arsenic or fluoride alone. In some cases antagonistic effects were noted following co-exposure to arsenic and fluoride. Arsenic and fluoride concentration increased significantly on exposure. Interestingly, their concentration decreased significantly on concomitant exposure for 8 weeks. However, the group which was administered arsenic for 4 weeks followed by 4 weeks of fluoride administration showed no such protection suggesting that the antagonistic effect of fluoride on arsenic or vice versa is possible only during interaction at the gastro intestinal sites. These results are new and interesting and require further exploration.

Keywords: Sodium *meta*-arsenite; Oxidative stress; Hematopoietic system; Hepatic; Sodium fluoride; Cell injury; Arsenic and fluoride concentration; Mouse

1. Introduction

Arsenic and fluoride are widely distributed in nature in many forms and their compounds are being used extensively. Occupational exposure to arsenic mainly occurs in the production and use of pesticides, burning of coal and wood treated with arsenic. The main source of arsenic exposure for the general population is

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food and ingestion of drinking water with high level of arsenic [1]. Fluorine is not freely found in nature. Fluorine in drinking water is totally in ionic form and hence it rapidly, totally and passively pass through the intestinal mucosa and interferes with major metabolic pathways of the living system. Fluoride in small doses has remarkable prophylactic influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis [2]. Fluoride enter the body through drinking water, food, toothpaste, mouth rinses, and other dental products; drugs and fluoride dust and fumes from industries using fluoride containing salt and or hydrofluoric acid [3]. In India, 90% of village population and approximately 70% of urban population is dependent on ground water as a major source for drinking water, which is contaminated with soluble inorganic and organic materials. Millions of people are currently at risk all over the world because they drink water containing carcinogenic amounts of arsenic and fluoride [4]. Permissible limit of arsenic in water is 10 μg/l and for fluoride it is 1 mg/l as per WHO guidelines. In West Bengal, the arsenic concentration in some tube wells is as high as 3400 μg/l [5].

Although several hypotheses have been proposed, the exact mechanism of arsenic and fluoride toxicity has not been clearly defined. There are however, studies, which suggest that trivalent arsenic compounds can inhibit various enzymatic pathways including glycolysis and the tricarboxylic acid cycle by binding to sulphhydryl groups of enzymes while pentavalent arsenic can uncouple mitochondrial oxidative phosphorylation [6]. Biochemical assays revealed that the functions of nearly 200 enzymes are affected by arsenic exposure [6]. Other biochemical alterations after arsenic exposure include transcription factor activity [7], oxidative stress products such as lipid peroxides and malondialdehyde (MDA) [8]. Haem biosynthesis pathway is influenced by arsenic exposure in animals and humans [6,9]. Fluoride is an anion with a rather small molecular weight. It shows its effect on the organism by combining with calcium (II) ions (Ca^{2+}) and causing a severe hypocalcaemia. On the other hand, it is named a calcium ionophore as it enhances the transport of Ca^{2+} ions into cells [10]. Some studies revealed that fluoride induces excessive production of oxygen free radicals [11], and might cause the depletion in biological activities of some antioxidant enzymes like superoxide dismutase (SOD), catalase and, glutathione peroxidase (GPx) [12]. However, the manner in which the whole body effects are produced is still unclear. Efforts to prevent and treat fluorosis by therapeutic measures too have had only limited success [13]. Recently the interaction of arsenic and fluoride has received considerable attention because arsenic is a well known metalloid which generally exists as arsenites (As^{3+}) and arsenates (As^{5+}) whereas, fluoride is an anion and interaction between cation and anion could be of interest. Simultaneous exposure to arsenic and fluoride is an emergent endemic disease in China and few other neighboring countries including India [14,15]. There is however, paucity of experimental data on the interactive effects of sodium arsenite and sodium fluoride when administered concomitantly, on the major organs. Toxic effects of arsenic and fluoride when administered individually on various biochemical parameters are known [16,17] however, there is relatively no conclusive experimental evidence if the combined exposure will led to synergistic or antagonistic effects in animals [15,18]. Zhang et al. [14] suggested that the toxicological effects of fluoride could be enhanced by arsenic while, contradictory results suggesting independent, synergistic and antagonistic effects in different animal models too have been reported [18–20]. Combined arsenic and fluoride interaction through drinking water is thus not an exceptional condition and may lead to antagonism or synergism. With the use of fluoride containing toothpastes and mouth washes, addition of fluoride to water and in some countries there has been reports of high fluoride and arsenic concentration in ground water, such exposure are possible.

The present study thus was planned to assess the toxic effects of arsenic and fluoride individually, and during co-exposure on biochemical variables suggestive of alterations in hematopoietic, hepatic and renal oxidative stress, and arsenic and fluoride concentration in blood and soft tissues.

2. Experimental

2.1. Chemicals and reagents

Sodium *m*-arsenite ($NaAsO_2$, molecular weight 129.9) and sodium fluoride (NaF, molecular weight 41.99) were procured from Sigma Chemical (USA). All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Ultra pure water prepared by Millipore (New Delhi, India) was used through out the experiment to avoid metal contamination and for the preparation of reagents and buffers used for various biochemical assays in our study.

2.2. Animals and treatment

All experiments were performed on healthy, adult male mice of Swiss strain, weighing approximately