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Interactions of Arsenic with Fluorine, Selenium, Barium, and Strontium in Human Hepatic Cells

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Arsenic (As) is a ubiquitous trace element present in various compounds throughout the earth's crust. Many arsenic compounds are known to be toxic, and the exposure of humans and animals especially, and ecosystems in general, to arsenic remains of international concern (Harrison and Rapsomanikis, 1989). The general population is exposed to inorganic and organic arsenic through drinking water, air, food, and beverages. The ingested or inhaled inorganic arsenic through medicinal, occupational and environmental exposures is well-documented as a human carcinogen of skin and lung (IARC, 1987). In addition significant associations between ingested arsenic and malignant neoplasms of the liver, bladder, and kidney among residents in the endemic area of chronic arsenicism in Taiwan were recently reported by many researchers (Chen et al., 1985 and 1986; Wu et al., 1989).

More than at least several hundred thousand people in China are suffering from arsenic poisoning. We found high concentrations of fluorine (F), barium (Ba) and strontium (Sr) as well as As in well water in a village (Wuyuan), the Inner Mongolian Autonomous Region in China during 1997 and 1998 (Fig. 1). Most of persons chronically exposed to high levels of As from drinking water have caused skin alterations such as keratosis, hyperpigmentation and depigmentation, while skin cancer has not been induced by arsenic yet. The concentrations of As, F, Ba and Sr in well water in the village, the Inner Mongolian Autonomous Region in China during 1997 and 1998 were much higher than the other elements such as Se, copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). The As concentration of drinking water sample in the village, the Inner Mongolian Autonomous Region in China was 0.4 mg/L. In some areas where chronic arsenic poisoning such as skin cancer has occurred, levels exceeding approx. 1 mg / L have been recorded in well water (WHO, 1981). On the other hand F and Ba concentrations were approx. 1.3 mg/L and 0.2 mg/L, respectively. Many Individuals are supplied with drinking water with a natural fluoride content of 1 mg/L or more (WHO, 1984). Studies of the water quality in cities in the USA have revealed levels of Ba ranging from a trace to approx. 10 mg/L (WHO, 1990). The interactions of As with F, Ba, Sr or other elements on the inhabitants have been unknown, and information regarding the interactions of As with F, Ba,

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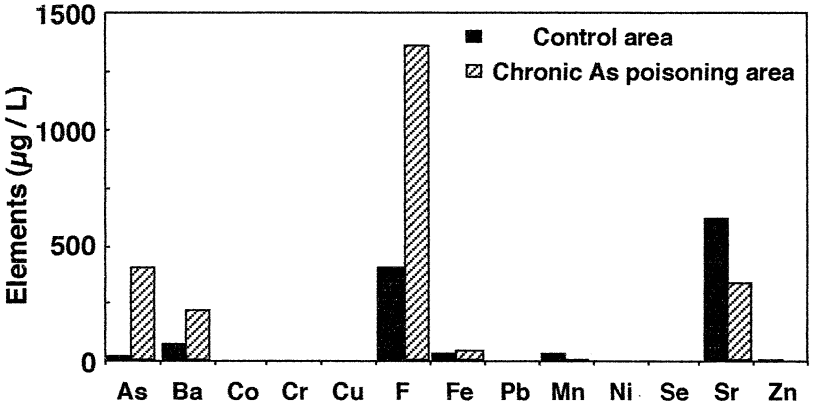

Figure 1. Concentrations of each element in drinking water in a village, the Inner Mongolian Autonomous Region in China.

Sr and other elements in vitro is also scarce. Interaction is that marginal effects and concentrations of a toxic element change under a condition containing several toxic elements and that the metabolism and toxicity alter. The protective effect of simultaneous Se administration on acute cadmium toxicity or indium (In) administration on acute cadmium toxicity in vivo has been studied (Yoshikawa, 1968; Ohta et al., 1988). The purpose of this study is to examine interactions of As with F, Ba, Sr or Se in human hepatic cells.

MATERIALS AND METHODS

All chemicals used were of reagent grade. NaF, $BaCl_2$, $SrCl_2$ and Na_2SeO_3 were purchased from Wako Pure Chemical Industries, Osaka, Japan. As_2O_3 was from E. Merck, Darmstadt, Germany. Alamar Blue was purchased from Alamar, Sacramento, CA, USA. Minimum Essential Medium Eagle (MEM-E) was obtained from Sigma, St. Louis, MO, USA. Fetal bovine serum (FBS) and trypsin-EDTA were obtained from Gibco-BRL, Rockville, MD, USA.
Concentrations of As, Ba, cobalt (Co), chromium (Cr), Cu, F, Fe, lead (Pb), Mn, nickel (Ni), Se, Sr and Zn in well water in a village (Wuyuan), the Inner Mongolian Autonomous Region in China were measured with ICP-MS.
Chang liver (CCl-13) cells from non-malignant human tissues were obtained from American Type Culture Collection, Rockville, MD, USA. The cells were passaged once a week and cultured in MEM-E medium supplemented with 10% FBS. Exponentially growing cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C.

The hepatic cells were counted overnight using a Becton Dickinson CC-VI blood cell counter. Each value represents the mean of three experiments. The cells were plated at a density of 2 x 10^3 cells/200 μl/well in 96-well, flat-bottomed multiple well plates (Corning Laboratory Science Company, NY, USA) and were incubated overnight at 37°C. NaF, $BaCl_2$, $SrCl_2$ and Na_2SeO_3 were dissolved in H_2O. As_2O_3 was added to a polypropylene tube containing 2N NaOH, heated

and dissolved in a heating block at 95°C for 3hr. The solution was diluted (1:50) with H_2O. Two experiments were carried out. Each (2 µl) of them alone was added into the culture media to final concentrations of 100 µM, and to examine each interaction of As with F, Ba, Sr or Se, 1 mM As plus 0.5 - 10 mM F, Ba, Sr or Se were prepared and 2 µl of them were added into the culture media.

The pH of the media after adding the chemicals remained unchanged. After heavy metal exposure, cells were incubated at 37°C for 24 hr. The stock solution of Alamar Blue was aliquoted and kept in the dark at 4°C. Upon the termination of heavy metal exposure, cells was rinsed three times with phosphate buffered saline (PBS) and incubated at 37°C for three hours in alamar blue containing media (10% Alamar Blue in MEM-E media) according to the manufacturer's instructions. Alamar Blue assay was performed by the method with slight modifications of Ahmed et al. (1994). Alamar Blue contains an oxidation-reduction (Redox) indicator. Cellular proliferation induces chemical reduction of the media which results in a change in Redox color from the blue to red. The intensity of red color reflects the extent of cellular proliferation. The emission and excitation spectra of Alamar Blue growth indicator were determined on a fluorescence spectrophotometer model Cyto FluorII Series 2000 (PE Biosystems, Foster City, CA, USA). An excitation wavelength of 530 nm (25 nm bandwidth filter) was used and emission was at 590 nm (50 nm bandwidth filter). Average readings of 3 wells for each mixture were used. This will accurately determine the reduced state (specific absorbance) which reflects specific level of proliferation. Statistical evaluations in some experiments were performed by the Student's t-test using the Statview II program on a Macintosh computer, $\alpha = 0.05$.

RESULTS AND DISCUSSION

We investigated each interaction of As with F, Se, Sr and Ba in human hepatic cells. Figure 2 shows interactions of As with F, Se and Sr in human hepatic cells. Although 5 to 100 µM F alone had no effect on the proliferation of human hepatic cells, there were significant increases in cell viability after exposure to 10 µM As + 100 µM F as compared to As alone (Fig 2A). Significant increases in cell viability were not observed below the concentrations of 10 µM As + 100 µM F. The concentrations of 10 - 100 µM Se alone decreased in cell viability significantly as compared with control (Fig. 2B). For the concentrations of 10 µM As + 50 µM Se and 10 µM As + 100 µM Se, a significant decrease was found as compared to As alone. For the10 µM As + 5 µM to 100 µM Sr, no significant changes were observed as compared to As alone (Fig 2C) as well as the As+Ba (data not shown). Figure 3 shows morphological changes in human hepatic cells caused by As and 3 mixtures containing As (10 µM As + 100 µM Se, 10 µM As + 100 µM F and 10 µM As + 100 µM Sr). The mixture with 10 µM As and 100 µM F induced the proliferation of human hepatic cells in comparison with As, while the mixture with 10 µM As and 100 µM Se inhibited the spreading of human hepatic cells against As toxicity. The other mixtures (10 µM As + 100 µM Sr) had no effect on the proliferation of human hepatic cells against As toxicity. The data of the morphological changes were similar with

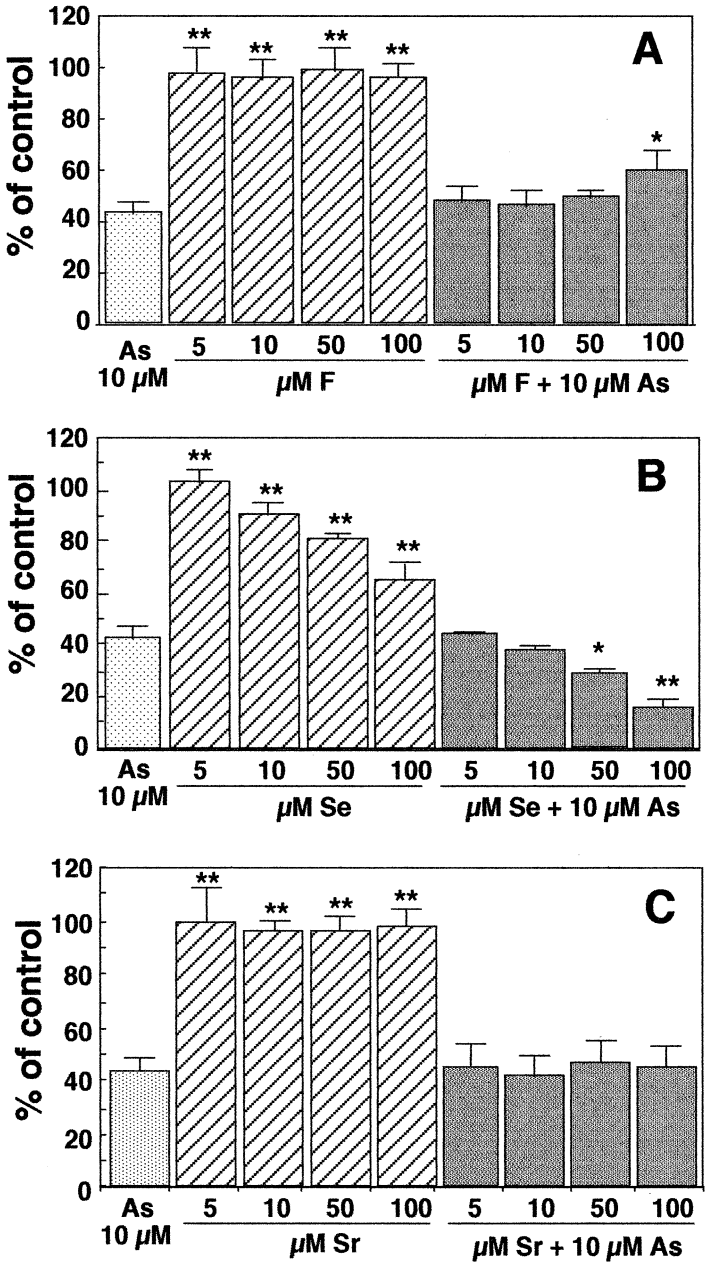

Figure 2. Effects of As alone and 3 mixtures containing As (10 μM As + 5 to 100 μM F, 10 μM As + 5 to 100 μM Se and 10 μM As + 5 to 100 μM Sr) on human hepatic cells in vitro. Data expressed as mean ± SD (n=3). * $P< 0.05$ and ** $P< 0.01$ in comparison with cells exposed to As alone.

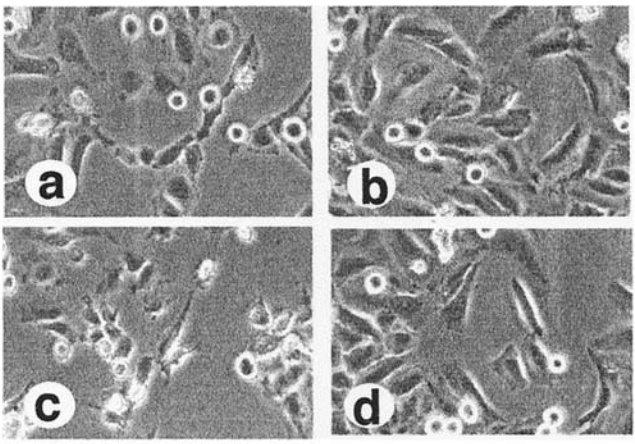

Figure 3. Interactions of As with F, Se and Sr. Phase-contrast micrographs of human hepatic cell cultures treated with As alone (a) and 3 mixtures containing As (b; 10 μM As + 100 μM F, c; 10 μM As + 100 μM Se, d; 10 μM As + 100 μM Sr)(100X). The cultures were incubated with As alone and the 3 mixtures for 24h.

those by using Alamar Blue (Fig 2). These results indicated that the high concentration of F protected against As toxicity in human hepatic cells and that Se accelerated As toxicity against human hepatic cells.

At least 3,000 people in Guizhou Province in southwest China are suffering from severe arsenic poisoning (Finkelman et al., 1999). The primary source of the As appears to be consumption of chili peppers dried over high-arsenic coal. More than 10 million people on Guizhou Province and surrounding areas suffer from dental and skeletal fluorosis by F (Finkelman et al., 1999).

The excess F is caused by eating corn dried over burning driquettes made from high-fluorine coals and high-fluorine clay binders. Se often has a protective effect on the animal studied (Naganuma et al., 1983). The protection seems to be due to the precipitation in vivo of the element in the presence of Se which inhibits the toxic effect of the element considered such as As, mercury, copper, platinum (Parizek and Ostadalova, 1967; Hill, 1974; Levander, 1977). These reports in vivo were contrary to our results on human hepatic cells in vitro. Several hundred cases of acute or subacute barium poisoning occurred in the Kiating district of China, where table salt contained a large amount of Ba. The victims suffered sudden attacks of paralysis, ranging from mild to severe, paraesthesia, and cardiac symptoms, but recovery was usually rapid (Allen, 1943). In conclusion our results indicated that the high concentration of F protected against As toxicity in human hepatic cells and Se accelerated As toxicity against human hepatic cells. This study regarding the interaction of As with F, Se, Ba and Sr will be helpful in understanding toxic effects of the heavy metals on certain organs and will lead to a better understanding of the chronic arsenic poisoning in China.

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