GENERAL AND BEHAVIORAL TOXICOLOGICAL EFFECTS OF SUBCHRONIC INORGANIC ARSENIC AND FLUORIDE TREATMENT IN ADULT WISTAR RATS

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Oral exposure of humans by excess amounts of arsenic and/or fluorine may cause disturbances of the nervous system. In the present study, such exposure was modelled in rats, with examination of general and behavioral endpoints. Seven weeks old adult male SPF Crl:WI BR Wistar rats (160±20 g, 4 groups of 12 rats each) were treated with sodium (meta)arsenite (10 mg/kg b.w.; *As*), sodium-fluoride (5 mg/kg b.w.; *F*) and their combinations (*As+F*) per os by gavage, 5 days in a week, once a day for 6 weeks. An untreated control group was also used (*Control*). General toxicological parameters (body weight gain, food and water consumption) were measured daily. Behavioral investigations (rota-rod and open field) were done in the 4th and 6th weeks of treatment. Weekly body weight gain was significantly reduced in the *As* (vs. *F*) and *As+F* (vs. *Control* and *F*) groups from the first week onwards. This difference was seen during the whole treatment period, and was more prominent from the second week on (*As* and *As+F* vs. *Control* and *F*). As, but not F, affected the relative weight of the liver, spleen and kidneys. Food and water consumption in the *As* and *As+F* groups was significantly reduced vs. *Control* and *F,* while a non-significant increase of water consumption was seen in group *F*. In the open field test, *As* and *As+F* caused significant decrease in rearing and ambulation, and increase in immobility and local activity, vs. *Control* and *F*. In the rota rod performance, no noteworthy change was observed. In the treatment scheme applied, significant effects on both general and behavioral endpoints by arsenic, but not by fluorine, were detected, which underlines the risk from environmental exposure.

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Introduction

Drinking water can have positive health effect by supplying essential nutrients, but also negative effect if toxic components are present. The population of Hungary and some other countries (Bangladesh, West Bengal in India, Vietnam, Taiwan, Argentina, Chile, Mexico, Brazil and Romania) may be at risk by inorganic arsenic, as geological contaminant in the drinking water[1]. Both arsenic (As) and fluoride (F) are present in groundwater at high concentration in India, China, Mexico, Argentina and Bangladesh[2]. In China and India, endemic fluorosis has been reported[3]. In the exposure of humans to both As and F, inhalation, ingestion and skin contact can each have an important role.

Fluoride is an essential element, necessary for the development of bones and teeth[2]. In case of excess exposure, however, it can be neurotoxic. The general population might be exposed to F directly by drinking water taken from certain subsurface sources, or through the food chain by plants accumulating F[4]. The automotive industry has used F for surface treatment in plastic fuel tanks for many years. Other important F sources in occupational exposure are aluminium metallurgy and microelectronics. F is able to cross the blood-brain barrier that can cause biochemical and functional changes in the nervous system[4]. In case of F intoxication, dental and skeletal fluorosis (brown coloration of the teeth, brittle bones and bone deformities, limited movement of joints, intense calcification of ligaments, muscle wasting etc.) and neurological deficits can occur[5].

To our present knowledge, As is a micronutrient but in higher concentration it is a poison[6]. As is present in the rocks and soil, but beyond natural geological sources, As contamination can occur from mining, smelting and from burning of coal with higher As content[6]. In the soil, As may also occur after uncontrolled use of pesticides containing As (using these pesticides is now banned). The most toxic form of As is inorganic arsenite (As^{III}). Arsenate (As^{V}), the other inorganic form is much less toxic and organic As is mostly harmless except for a few compounds like phenylarsenic acid[9].

Exposure to chronic inorganic As causes cardiovascular, hepatic and renal diseases (cancer in kidney, liver and lungs) furthermore central and peripheral nervous system abnormalities. Headaches, weakness and mental confusion were also reported[7].

Numerous reports are found in the literature regarding individual toxicity of As and F, but there is not enough information about the effects from combined exposure to these elements[2]. Some reports show that As and F poisoning are co-existent in certain countries and that the toxicological effects of these elements possibly enhance each other. On the other hand, some results suggested that there is an antagonistic effect between As and F[8].

The aim of the present study was to give a model for the individual and combined exposure to F and inorganic As, as neurotoxic compounds, through the gastrointestinal tract.

Materials and Methods

Animals and treatment

Young adult – 7 weeks old, body weight 160±20 g – SPF Wistar rats (Crl: WI BR) were obtained from Toxi-Coop (Budapest). The animals were kept in polypropylene home cages (3 rats/cage) under GLP-equivalent conditions (12-12 hour light/dark cycle with light on at 06:00; temperature 22-24 °C, 30-60% relative humidity). The rats had free access to drinking water and rodent chow (Ssniff R/M-Z+H).

The experiment was started with 48 rats, and the animals were distributed randomly to four groups of 12 animals each according to their body weight. After one week of acclimatization, 3 groups were treated with sodium (meta)arsenite ($NaAsO_2$, 10 mg/kg b.w., equivalent to 5.8 mg/kg b.w. As; group *As*), sodium fluoride (NaF 5 mg/kg b.w., equivalent to 2.3 mg/kg b.w. F; group *F*) and their combinations (group *As+F*) per os by gavage, 5 days a week once a day for 6 weeks. The control group received distilled water (*Control*). All chemicals were purchased from Sigma Aldrich.

General toxicological investigation

During the treatment period, body weight along with water and food consumption, was measured every day. At the end of the 6 weeks treatment, the animals were dissected and organ weights were measured (relative organ weights related to 1/100 body weight).

Behavioral investigations

Open field and rota rod tests were done in the 4th and 6th week of treatment. The rats' spontaneous locomotor activity was investigated in an open field box (Conducta 1.0 System, Experimetria Ltd., Hungary). The animals were placed into the centre of the box, and the motility parameters – ambulation distance, time and count; rearing time and count; local time and count; immobility time and count – were measured in a 10 min session. Motor coordination of the animals was tested by rota rod (ROTA-ROD for rats 47700, Ugo Basile, Italy). During the experiment the rats had to stay on the top of the rod that was accelerating evenly from 2 to 10 rpm in 300 sec. The time span for which an animal remained on the rod without falling off was measured.

The data were analyzed by one-way ANOVA. Post hoc analysis of group differences was performed by Scheffe's test, with probability level at $p<0.05$.

Results and Discussion

General toxicological parameters

During the treatment period the general condition of the animals was observed. In the *Control* and *F* groups no considerable changes were seen. In contrast, the rats in group *As* had decreased appetite from the second week of treatment, were depressed, showed less activity in their cages, and their fur was rough, dry and off-white in colour. Further it was observed that in the *As+F* group the same changes occurred two weeks later, in the 4th week of the treatment. This difference was present also in the behavioral results but was not significant.

Food and water consumption in the *As* and *As+F* groups was significantly reduced (vs. *Control* and *F*; Fig. 1a and 1b; noted that in the 4th week, there was a significant difference between *As* and *As+ F* groups). The significant reduction of food consumption in the *As* group vs. the *As+F* group in the 4th week may show that F treatment (5 mg/kg b.w.) caused an antagonistic effect on the As treatment[2]. In contrast to other studies, the animals' increased water consumption in *F* group was not significant vs. other groups.

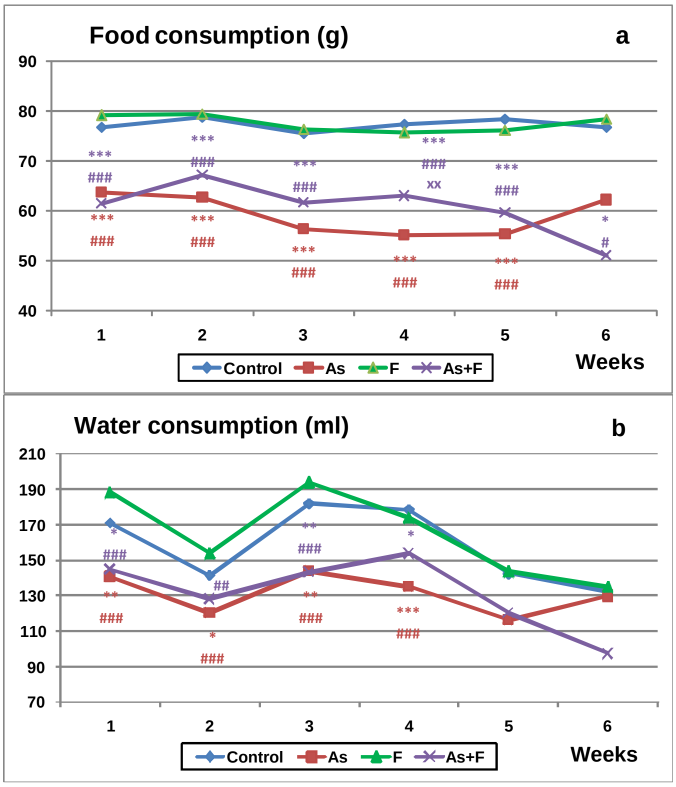

Figure 1. Food (a) and water (b) consumption of the rats during the 6-weeks treatment.

*, **, *** p<0.05, 0.01, 0.001 *As, As+F, F* vs. *Control;*
#, ##, ### p<0.05, 0.01, 0.001 *As, As+F* vs. *F;*
x p<0.05 *As* vs. *As+F.*

Weekly body weight gain was significantly reduced both in groups *As* (vs. *F*) and *As+F* (vs. *Control* and *F*), from the first week onwards. This difference was seen during the whole treatment period, and was more prominent from the second week on (*As* and *As+F* vs. *Control* and *F*; Fig. 2). The alteration of the body weight was directly proportional to the food consumption change.

Our results show that As treatment used in this study ($NaAsO_2$; 10 mg/kg b.w.) induced a significant body weight loss because of the reduced food consumption, which is in line with other studies[10,12]. On the other hand, F had no effect on the body weight.

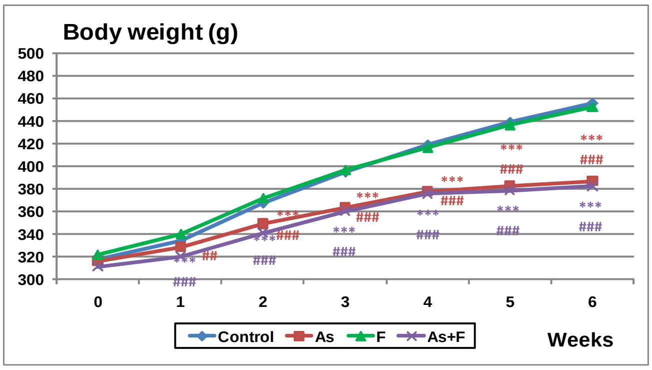

Figure 2. The effect of the 6-weeks treatment on the body weight gain of the animals.

*, **, *** p<0.05, 0.01, 0.001 *As, As+F, F* vs. *Control*;
#, ##, ### p<0.05, 0.01, 0.001 *As, As+F* vs. *F;*

Relative organ weights are shown in Table 1. In the *As* group, the relative weight of the liver, spleen and kidneys was increased significantly vs. *Control* and *F*. In the *As+F* group only the relative weight of spleen was increased significantly vs. *F*.

Table 1. Relative organ weights (related to 1/100 body weight) after the 6-weeks treatment.

*, **, *** p<0.05, 0.01, 0.001 *As, As+F, F* vs. *Control*;
#, ##, ### p<0.05, 0.01, 0.001 *As, As+F* vs. *F;*

GROUPS	*Control*	*As*	*F*	*As+F*
Brain	0.38±0.08	0.48±0.07	0.45±0.05	0.43±0.04
Thymus	0.12±0.02	0.12±0.02	0.12±0.02	0.10±0.02
Heart	0.25±0.01	0.28±0.05	0.25±0.02	0.28±0.03
Lungs	0.39±0.04	0.49±0.08	0.42±0.03	0.41±0.06
Liver	3.03±0.16	3.81±0.57 * #	3.16±0.17	3.30±0.14
Spleen	0.21±0.03	0.29±0.03 ** ###	0.18±0.02	0.24±0.02 #
Kidneys	0.59±0.06	0.71±0.07 * #	0.58±0.05	0.62±0.04
Adrenal Glands	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.01

After absorption, arsenic is distributed to organs or tissues, mainly to the liver where it undergoes methylation. The more toxic As^{III} binds thiol or sulfhydryl groups in thiol containing proteins of the liver, kidneys, spleen, lungs and gastrointestinal tract, and in keratin-reach tissues. As is eliminated through the kidneys rapidly[2,9]. In our work the increased weight of liver, kidney and spleen suggested that the arsenic accumulated in, and caused damage to, these organs[9].

Behavioural toxicological investigations

Open field test

The effect of arsenic and fluorine on open field (OF) motility was quite different. Both in the 4th and the 6th week, *As* and *As+F* caused significant decrease in motility: time and event count of rearing and ambulation decreased while the same indicators of immobility and local activity increased, vs. both *Control* and *F*. In contrast, the data of group *F* were nearly identical to those of *Control*, and the data of *As+F*, to *As* (Fig. 3, Fig. 4).

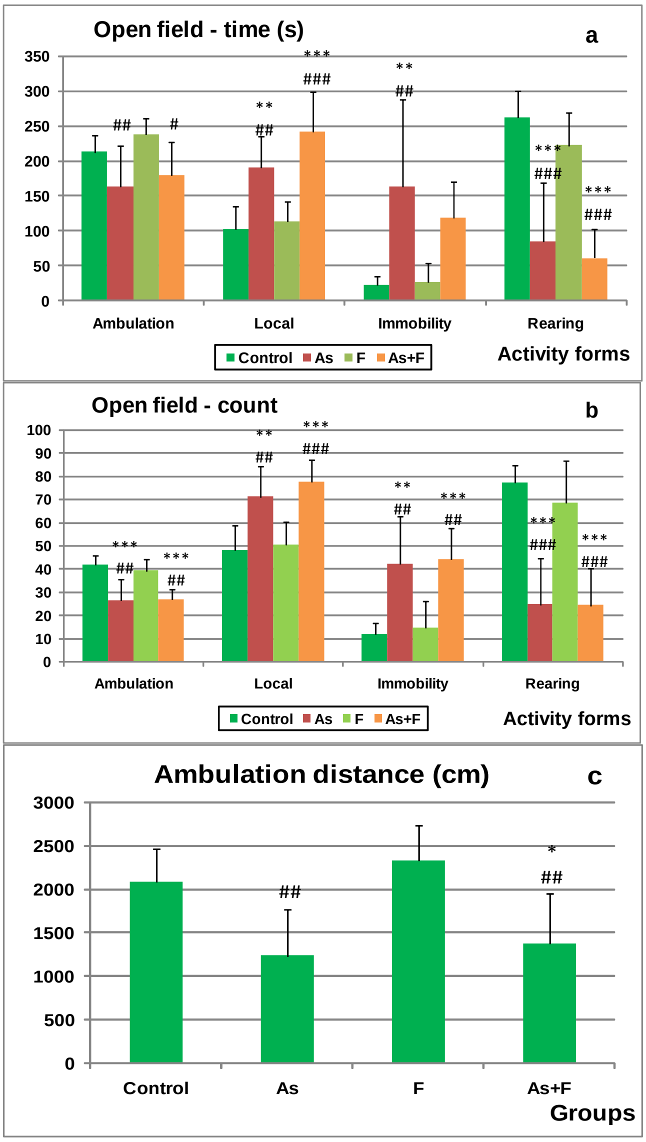

Figure 3. Open field test in the 4th week of the treatment. Time (a) and count (b) of the activity forms, and ambulation distance (c).

*, **, *** p<0.05, 0.01, 0.001 *As, As+F, F* vs. *Control*;
#, ##, ### p<0.05, 0.01, 0.001 *As, As+F* vs. *F;*

In the 4th week *As* and *As+F* caused significant decrease in ambulation distance vs. both *Control* and *F*, in contrast in the 6th week there was no significant difference between them (Fig. 3c).

To investigate the animals' movement is very important and useful in various behavioral studies. As can cross the blood-brain barrier and accumulates in the brain that may explain the central nervous system impairments. In rats treated with inorganic As, abnormal behavior (motility changes) and decrease of locomotor activity were observed which is consistent with earlier studies[12]. The decreased ambulation activity is possibly the result of an impairment of dopaminergic neurotransmission[10].

Rota rod test

Rota rod test in the 4th and 6th week of treatment revealed no noteworthy difference in the rats' performance. The basis of this test is that impairment of the dopaminergic system causes decreased motor skills. Intake of F in high concentration causes damage to the musculoskeletal and nervous system.

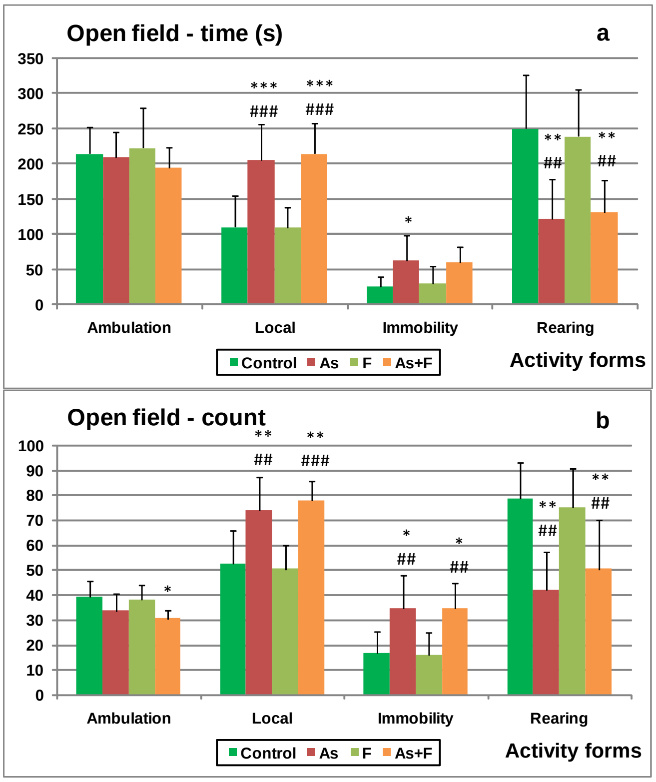

Figure 4. Open field test in the 6th week of the treatment. Time (a) and count (b) of the activity forms.

*, **, ***   p<0.05, 0.01, 0.001 *As, As+F, F* vs. *Control*;
#, ##, ###   p<0.05, 0.01, 0.001 *As, As+F* vs. *F;*

Some previous studies have shown a shortening of rota rod endurance time in F treated rats, while other studies indicated no changes in the motor coordination after such treatment[11]. Impaired motor coordination and concentration are common central nervous system manifestations in As exposure[12]. However, in our study there was no difference in the *As* treatment group vs. other groups.

Conclusion

In the treatment scheme applied, significant effects on both general and behavioral endpoints by As, but not by F, were detected. In the groups receiving arsenic, significant increase of the time spent with local activity and immobility, and significant decrease of vertical activity, indicated that the central nervous system was affected. Furthermore, F might have an effect on As toxicity.

Our results underline the risk from environmental inorganic arsenic exposure, and, together with the lack of effect of fluoride (contradicting available literature data) point to the need of further investigation in this field.

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