EFFECTS OF ARSENIC OR/AND FLUORIDE
ON MINERALIZED TISSUES OF THE RAT

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SUMMARY: Nuclear proton microprobe and histomorphometry were employed to study arsenic and fluoride distribution and effects on the mineralized tissues (teeth and bone) of rats. The results revealed that 1) fluoride (100 mg/L for 10 weeks) caused typical dental and skeletal fluorosis in rats, similar to those seen in humans; 2) no obvious effects of arsenic (30 mg/L for 10 weeks) on the mineralized tissues in rats were observed; 3) arsenic and fluoride in combination reduced the body weight of the rats significantly, presenting a synergistic effect; 4) arsenic insignificantly reduced fluoride depositions in the mineralized tissues; 5) no significant effects of arsenic were found on fluoride toxicity to the mineralized tissues in rats.
Key words: Arsenic; Combined effect; Fluoride; Mineralized tissue; Rat.

Introduction

Epidemiological surveys revealed that an excessive amount of arsenic in drinking water, or in the food polluted by coat-burning, might elevate the occurrence and prevalence of endemic fluorosis.[1,2] Experimentally, the evidence, that arsenic enhanced the effect of fluoride on the excretion of urine hydroxyproline in rats, suggested a possible additive or synergistic toxicity to mineralized tissues.[3]

A field survey on oral diseases showed, however, that the patients with combined toxicosis from arsenic and fluoride, due to drinking water, presented lower occurrence of dental fluorosis than those with fluorosis alone.[4] An antagonism between arsenic and fluoride on occurrence of skeletal fluorosis in rabbits was also possible.[5]

The nature of a possible interaction between arsenic and fluoride is little understood but in China, because of a potential role in a major health problem, it requires elucidation.

This study employed a 2 x 2 factorial design, modern proton microprobe and histomorphometry, and observed arsenic and fluoride distributions as well as their effects on the mineralized tissues.

Materials and Methods

Reagents: Arsenic trioxide (As_2O_3, BDH, AnalaR) and sodium fluoride (NaF, BDH, AnalaR) were administered in drinking water, and the concentrations were expressed as mg/L of arsenic (As) and fluorine (F).

Animals and treatment: 40 male Wistar rats, weaned two weeks, average weight 73.4 ± 25.4 g, were randomly divided into four groups. Group A received 30 mg As/L, Group B received 100 mg F/L, Group C 30 mg As + 100 mg F, and Group D de-ionized water (the control) in their drinking water *ad libitum.*

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The animals were fed a standard laboratory rat diet containing 0.35 mg As/kg, 18 mg F/kg, 1.2 mg Ca/kg and 0.75 mg P/kg. The weight of each rat was recorded weekly. The mean amount of consumed water was measured twice a week. After 10 weeks, the animals were killed by carbon dioxide inhalation. Samples of appropriate tissues were removed immediately, and stored in buffered formalin at 4°C.

Grading of incisor fluorosis: The appearance of the labial surface of erupted incisors was examined by three independent examiners closely with the naked eye or under a dissecting microscope following paper drying, and also recorded on photographs for further assessment. The changes in the appearance were graded as follows: I, yellow or orange and translucent; II, transverse, regular fine striations of alternating brown and white bands; III, irregular striations, the white space between the brown striations becomes wider and broken, but the brown striations are still seen on more than 50 percent of the surface; IV, irregular brown striations, but on less than 50 percent of the surface; V, overall white or chalky appearance with tip worn off or the enamel pitted.

Measurements of longitudinal length of incisor: Longitudinal length of incisors at three zones (the apical end of the incisor to the start of the white opaque zone, secretory stage of amelogenesis; the white opaque zone, maturing stage; the pigmented zone, matured enamel) on the labial surface of enamel were measured in a microscope equipped with a micrometer scale.[6,7]

Measurements of mandibular ramus: The mean length of mandibular ramus was determined by averaging the distances from the articular surface of condyle to the tip of the mesial cusp of the first molar and to the deepest concavity on the inferior border of the mandible with a micrometer scale.[8]

Determinations of arsenic and fluorine distribution by proton microprobe: One lower incisor and distal femur from each rat were embedded in epoxy resin and cut into two halves longitudinally with a diamond disc in a dental handpiece. Arsenic and fluorine profiles were determined by proton microprobe.[9,10] Three zones of an incisor were scanned across the middle line of each zone, and two zones of the femur were scanned, one across the epiphysis containing cancellous bone (trabeculae), and another across the diaphysis consisting of compact bone (cortex).

Histomorphometry: The fixed mandibular incisors were decalcified in 7% EDTA containing 7% sucrose and embedded in Epon. The incisors were cut into 3-4 pieces across each surface zone reference point, one micrometer thick sections were cut and stained with toluidine blue. Mean thickness of enamel (MTE) and mean height of ameloblast layer (MHA) in the white opaque part, trabecular bone volume (TBV) and mean thickness of cortex (MTC) were measured in a light microscope equipped with ZEISS II integrating eyepiece graticule.

Statistical analysis: No significant differences existed between the measurement variability within the same group. Therefore all measurements from the animals within each group were pooled and averaged. Interaction between groups was analyzed by two-way method. The interaction value (IV) for each examined parameter is given by calculation of the formula (mean value of Group D + mean value of Group C) - (mean value of Group A + mean value of Group B). Kappa

from comparisons between grading of macroscopic appearance of incisors by three independent examiners showed substantial agreement (7.6, 8.2 and 8.8 respectively). A X^2-test method for comparison of proportions was used for the data from grades of dental defects. All the statistic analyses were done by microcomputer using statistics software package (Statistics, SAS, 1986).

Results

Changes in body weight: Rats from the As-F mixed group had obvious lower final body weight and showed significant interaction (Table 1).

Changes in appearance of incisors: The labial appearance of incisors was similar for all rats in the As-exposed group (A) and identical to those of the control group. Typical dental fluorosis was recorded both in the F-exposed group (B) and the mixed group. However, no significant difference between the two groups in the distribution and constituent ratio of the grade was shown ($X^2 = 1.06, 0.5 > p < 0.75$).

Changes in longitudinal length of incisors: A significant increase in the length of the opaque part and an obvious decrease in the length of the pigmented part, both in the F-exposed group and the mixed group, were noted but showed no significant interaction (Table 1).

Changes in mandibular ramus: The length of the ramus in the mixed group showed insignificant decrease, and no significant interaction between the two chemicals was shown (Table 1).

Alterations in histomorphometric parameters: Decreased MTE and MHA as well as increased TBV were recorded in the F-exposed group. No interaction was found between the two chemicals (Table 2).

Fluoride contents in incisors: No differences were observed in fluoride contents in enamel and dentine between the As-group and the control group. However, the contents of fluoride in the As-F mixed group were significantly higher than those in the As-group and the control, but insignificantly lower than those in the F-exposed rats. No significant interaction between the two chemicals was shown (Table 3).

Fluoride contents in femur: The levels of fluoride in the compact and cancellous bone of femur from the As-F mixed group were similar to those from the F-exposed group (Table 3).

TABLE 1. Final body weight, length of incisor and length
of mandibular ramus of the rat (M±SD, n=10)

| Group | Body weight (g) | Length of incisor (mm) | | Length of ramus (mm) |
		Opaque	Pigmented	
A(As)	370.0 ± 27.4	9.4 ± 0.62	12.7 ± 0.82	16.25 ± 0.38
B(F)	371.6 ± 21.2	10.1 ± 0.48*	11.5 ± 0.85*	16.35 ± 0.46
C(As+F)	349.2 ± 29.2[a]	9.9 ± 0.45[b]	11.88 ± 1.0[c]	15.86 ± 0.27[d]
D(control)	388.4 ± 16.9	9.1 ± 0.56	13.2 ± 0.5	16.39 ± 0.16

* p<0.05 (compared with the control)
[a] IV = -4.0, p<0.05;   [b] IV = -1.3, p>0.05;   [c] IV = 0.88, p>0.05;   [d] IV = -0.35, p>0.05

TABLE 2. Histomorphometric parameters of measurements (M±SD, n=10)

| Group | Maturing enamel (μm) | | TBV(%) | MTC(mm) |
	MTE	MHA		
A(As)	134 ± 34.6	132 ± 25.4	28.4 ± 6.4	0.91 ± 0.06
B(F)	108 ± 28.3*	86 ± 21.6*	42.1 ± 5.6*	0.86 ± 0.08
C(As+F)	124 ± 24.6[a]	96 ± 24.4[b]	36.7 ± 6.8[c]	0.93 ± 0.14[d]
D(control)	146 ± 25.8	126 ± 23.3	27.6 ± 4.4	0.99 ± 0.08

* $p < 0.05$ (compared with the control)
[a] IV = 18, $p > 0.05$;   [b] IV = 6, $p > 0.05$;   [c] IV = -6.5, $p > 0.05$;   [d] IV = 0.05, $p > 0.05$

TABLE 3. Fluoride contents in the incisor and bone of the rat (M±SD, mg/kg, n=10)

| Group | Pigmented enamel | | Femur bone | |
	Enamel	Dentine	Cortical	Cancellous
A(As)	120.4 ± 29.7	265.9 ± 31.7	380.2 ± 64.3	324.8 ± 52.8
B(F)	840.0 ± 224.3	1718.5 ± 310.9	3148.3 ± 364.9	1624.6 ± 241.7
C(As+F)	757.8 ± 129.8[a]	1617.4 ± 408.3[b]	2986.6 ± 302.5[c]	1583.4 ± 198.6[d]
D(Control)	122.4 ± 24.5	285.8 ± 30.8	416.8 ± 78.2	386.9 ± 83.9

[a] IV = -80.2, $p > 0.05$; [b] IV = -81.2, $p > 0.05$; [c] IV = -125, $p > 0.05$; [d] IV = 20.9, $p > 0.05$

Discussion

Body weight gain during experimentation reflects body reactions to the toxicity of the stimuli: 5, 10 and 20 mg/L of arsenic as sodium arsenate administered in drinking water to rats for 12 weeks showed no significant effect on the body gain.[11] However, a significant decrease in body weight was observed in rats supplemented with 50 mg/L of arsenic as arsenic trioxide in drinking water for 10 weeks.[12] We administered rats with 30 mg/L of arsenic as the same type in drinking water for 10 weeks and observed a decreased trend of the body gain. The present study showed a dose-effect of arsenic on animal body growth.

Arsenic is of potential significance to the mineralized tissues because it is the next highest element to phosphorus in the Periodic Table. It therefore has similarities in valency and electron orbit. Still, an obscure relation of arsenic to the higher prevalence of dental caries and abnormal enamel was observed in arsenic-poisoned children.[13] However, experimental study failed to disclose dental caries-like defects associated with excessive intake of arsenic in experimental rodents.[11] The present study further confirms this earlier report: no obvious effects of arsenic on both dental and skeletal hard tissues were recorded in rats.

Absorbed inorganic arsenic in rats, like in humans, is initially inactivated in the liver to monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) which are excreted in urine along with a limited amount of unchanged inorganic arsenic.[14-16] Unchanged arsenic in the body mainly accumulates in hair and nails.[17,18] A little amount of arsenic exists in soft tissues or organs.[19,20] It seems that very few reports are available on the uptake of arsenic by mineralized tissues.[21] Negligible arsenic deposition in tooth and bone tissues[21] explains our negative findings: no detectable amount of arsenic was recorded by proton microprobe determination.

It is well recorded that fluoride causes dental fluorosis analogously both in humans and animals. Experimentally, it has been shown that the pathologic feature of dental fluorosis is characterized by subsurface porosity or hypomineralization in the enamel and dentine.[22-24] The visible clinical manifestations in human permanent teeth are the varying opacities.[25,26] In the present study a grading system for dental fluorosis was used, and the basic features of dental fluorosis in rats were observed to be similar in nature to those in humans, although shown as a series of loss of pigmentation.

An initial change was observed as transverse, brown fine lines. The space between the lines was so close that they could be identified only under the microscope after drying. The further change was that the space between the striations became wider and irregular with broken striations. The irregular, brown striations could be seen on more than 50 percent of the whole surface, and in a further grade on less than 50 percent. In the final grade, most of the striations disappeared, and the whole tooth had a chalk-like appearance with tips worn off or the enamel was fractured or pitted.

The labial surface of the continuously growing rat incisor normally has a thin brown pigmentation of iron, making the teeth look orange or brown. This thin layer of pigmentation is probably the product laid down normally by ameloblasts. The fluorosed enamel, as described above, underwent a series of loss of pigmentation and increased hypoplasia.

It was observed that the hypomineralized region, which expanded from the subsurface area towards the amelo-dentinal junction, occurred alternately in association with the striations in iron pigmentation on the enamel surface, namely in white bands.[27] The typical change, by which the incisor became increasingly chalky, can be explained as the result of periodical inhibition of enamel formation by fluoride. When blood fluoride reached a higher but stable level, perpetual inhibition of ameloblast activity occurred. The thin layer of iron pigmentation could not be laid down normally by the ameloblasts, and excessive fluoride was incorporated into the enamel simultaneously with the consequent deficient calcification of tooth tissue.

Regarding the mechanisms by which fluoride causes tooth defects, the striking feature observed has been that the ameloblasts, especially in maturing enamel, were dramatically inhibited by fluoride.[7] As seen in the present study, the fluoride toxicity to, or inhibition of, ameloblasts was directly reflected by the shortened height of the ameloblast layer, the lengthened maturation stage and the lowered eruption rate morphometrically.

Considering the combined effects of arsenic and fluoride, our results showed that the body weight of the rats in the As-F mixed group was significantly lower with significant interaction, revealing a synergistic effect between arsenic and fluoride on reducing body weight in rats.

The length of the mandibular ramus is a sensitive index reflecting toxicity to bone or bone growth.[8] However, like arsenic and fluoride alone, arsenic and fluoride in combination did not alter the index, and no significant interaction was shown, indicating that no obvious interaction exists between the two chemicals

affecting bone growth. Furthermore, the increased contents of fluoride in the femur bone in the As-F exposed animals were similar to those in the F-exposed animals alone, and the changes in trabecular bone volume in the mixed group was identical to that in the F-group, which indicates that arsenic insignificantly changed the deposition of fluoride, and did not alter fluoride effects on bone tissue (skeletal fluorosis), or *vice versa*. The conclusions are contradictory to those from a rabbit experiment which showed that arsenic probably reduced fluoride deposition in the bone and decreased the harmful effects of fluoride on bone.[5] The difference may be explained by the different experimental animals.

Similarly, arsenic did not significantly alter the content of fluoride in rat incisors, and did not obviously influence the typical damage or toxicity of fluoride to the dental tissues (dental fluorosis).

An initial survey in a Guizhou coal-smoke polluted type of endemic fluorosis area showed that arsenic may play a synergistic or additive effect on the prevalence of endemic fluorosis.[2] The present study, with dosages or proportions of arsenic and fluoride similar to the fluorosis area, failed to reveal this synergism in rats. Probably other chemicals in the coal-smoke emitted in the endemic fluorosis area influenced the effects of arsenic or fluoride, which are worth further study.

Acknowledgment

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