NEGLIGIBLE AMELIORATION BY ALUMINIUM SULPHATE ON SUBACUTE FLUORIDE-INDUCED ENZYMATIC ALTERATIONS IN GOATS

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SUMMARY: As part of a recent study on goats, alterations in enzymatic parameters were determined after oral administration for 30 days of 20 mg of NaF/kg bw/day to four healthy goats (Group 1) plus the same dose of NaF with 150 mg of aluminium sulphate/kg bw/day to another four healthy goats (Group 2). Significant decreases ($p < 0.01$) in acetylcholinesterase, phosphatases, and alanine aminotransferase activities were observed in both groups of goats, and a significant increase ($p < 0.05$) in aspartate aminotransferase activity was observed in only the Group 1 goats. The results indicated that concurrent administration of aluminium sulphate failed to show ameliorative action on the enzymatic alterations induced during subacute intoxication by NaF.

Keywords: Aluminium sulphate; Enzymatic alterations; Goat enzymes; Subacute fluoride toxicity.

INTRODUCTION

In the body, fluoride (F) can cross the cell membrane and affect various soft tissues like cardiac muscle, liver, skin, and erythrocytes,[1-3] causing impairment of soft tissue functions.[4-6] Studies on enzymatic alteration during F toxicity in avian species, cattle, sheep, rabbit, rats, and mice have been conducted, but controlled experiments with subacute F toxicity in goats are somewhat limited. Some salts of boron, calcium, magnesium, selenium, molybdenum, and aluminium have shown an ameliorative effect on plasma F levels by decreasing the gastrointestinal absorption of F in different mammalian species.[7-9] The ameliorative action of these salts is based on the clinical symptoms. Unfortunately, detailed studies on enzymatic changes during F exposure in combination with these ameliorative agents, particularly in goats, appear to be comparatively few. As already reported, our investigations have revealed that subacute exposure of goats to F alters their biochemical,[10] haematological,[11] and electrocardiographic parameters,[12] the F levels in plasma[13] and milk,[14] and causes significant oxidative stress.[15] The haematological parameters apart from the blood clotting time, the electrocardiographic changes, and the fluoride levels in plasma and milk were all ameliorated by the administration of aluminium sulphate but the biochemical parameters and the F-induced oxidative stress were not. As a part of that research, the present study is concerned with assessment of alterations in the enzymatic activities in the same goats after oral administration of NaF alone and in combination with aluminium sulphate.

MATERIALS AND METHODS

As reported in our recent publications,[10-15] eight healthy cross-bred goats 1.5-2.0 years of age were divided into two groups of four goats in each group. In

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Group 1, 20 mg of NaF (9 mg F)/kg/bw/day, and in Group 2 the same dosage of NaF plus 150 mg of aluminium sulphate hexadecahydrate (12.8 mg Al)/kg bw/day were administered orally in 100 mL of distilled water for 30 days. Plasma was separated for analysis of enzyme activities and the washed red blood cells were used for preparing the 1% haemolysate to assess the erythrocyte cholinesterase activity. Acetylcholinesterase (AChE) was assayed in the plasma as well as in the RBC (erythrocyte) lysate by the method of Voss and Sachsse.[16] The activities in the plasma of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH) were estimated as per the procedures described by Wootton.[17] Values on different days were compared to the pre-exposure (day 0) value of the same group by using Student's paired-t test, and a probability level of $p<0.05$ or $p<0.01$ was considered statistically significant.[18]

RESULTS

Details of the changes in the enzymatic profile of the Group 1 and Group 2 goats are presented in Tables 1–2 and 3–4, respectively.

Table 1. Effect of oral administration of NaF (20 mg/kg bw/day) daily for 30 days on enzymatic parameters (mean ± SEM) (n=4)

Parameter	Treatment days							
	0	1	3	7	14	21	28	30
RBC AChE (nmol thiol group formed/ min/mL)	6785.49 ±120.57	5382.95† ±375.56	4609.32† ±217.59	5685.91* ±356.79	7436.05* ±149.28	6713.81 ±491.39	7541.54 ±403.86	7523.96 ±346.55
Plasma AChE (nmol thiol group formed/ min/mL)	238.85 ±14.28	158.78 † ±9.22	71.28 † ±2.64	186.92 ±12.26	243.18 ±12.89	200.31 ±12.99	233.04 ±11.42	193.00 * ±4.63
ALP (nmol phenol produced/ min/mL)	120.41 ±24.59	76.11 ±14.07	35.83 † ±3.64	18.63 † ±1.91	15.92 † ±1.71	28.98† ±3.48	31.26† ±3.24	35.82 † ±1.68
ACP (nmol phenol produced/ min/mL)	0.763 ±0.08	0.586 ±0.14	0.583 ±0.05	0.395 † ±0.01	0.428 † ±0.01	0.511† ±0.00	0.898 ±0.14	0.563 ±0.03
ALT (nmol pyruvate formed/ min/mL)	19.91 ±1.21	27.86 * ±2.45	6.49† ±1.36	6.03 † ±1.49	4.03 † ±0.55	6.78 † ±0.67	8.51 † ±2.46	10.78 † ±1.86
AST (nmol pyruvate formed/ min/mL)	24.60 ±3.03	30.02 ±8.76	29.41 ±3.59	18.75 ±3.49	39.44 ±14.36	39.56 ±14.38	73.80 * ±17.96	42.84 ±9.46
LDH (nmol pyruvate reduced/ min/mL)	151.41 ±8.94	170.92 ±44.94	168.55 ±5.17	173.10 ±19.38	142.11 ±32.80	188.05 ±43.06	140.07 ±48.01	152.03 ±61.56

*† Significantly different as compared to pre-exposure (day 0) value at 5% ($p< 0.05$) and 1% ($p< 0.01$), respectively.
AChE: Acetylcholinesterase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase; LDH: Lactate dehydrogenase; nmol: nano moles.

Table 2. Effect on enzymatic parameters after the oral administration of NaF (20 mg/kg bw/day) daily for 30 days (mean ± SEM) (n=4)

Parameter	Post-treatment days		
	3	7	14
RBC AchE (nmol thiol group formed/min/mL)	8206.97 ± 528.19*	6272.90 ± 868.34	6469.01 ± 249.01
Plasma AchE (nmol thiol group formed/ min/mL)	282.67 ± 7.39*	204.90 ± 5.99	175.42 ± 12.26*
ALP (nmol phenol produced/ min/mL)	21.75 ± 3.40†	25.72 ± 6.19†	17.23 ± 3.96†
ACP (nmol phenol produced/min/mL)	0.560 ±0.10	0.332 ± 0.05 †	1.294 ± 0.31
ALT(nmol pyruvate formed/min/mL)	7.26 ± 0.97†	11.94 ± 0.49†	11.38 ± 3.06*
AST (nmol pyruvate formed/min/mL)	41.96 ± 12.62	26.34 ± 5.65	23.21 ± 1.87
LDH (nmol pyruvate reduced/min/mL)	134.39 ± 18.32	144.16 ± 13.46	136.98 ± 14.56

*† Significantly different as compared to pre-exposure (day 0) value at 5% ($p < 0.05$) and 1% ($p < 0.01$), respectively. AchE: Acetylcholinesterase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase; LDH: Lactate dehydrogenase; nmol: nano moles.

In Group 1, erythrocyte acetylcholinesterase (AChE) activity decreased significantly ($p<0.01$) from the pre-exposure level within 1 day and remained lower by 17% up to day 7 of exposure, after which it increased. In this group of goats the plasma AChE activity decreased significantly ($p<0.01$) by 71% after 3 days of exposure. Thereafter, the activity increased and the enzyme was maximally inhibited by 15-20%. Afterward the enzyme activity did not return to pretreatment values even on day 14 after cessation of treatment.

Also in the Group 1 goats, the plasma ALP activity decreased significantly ($p<0.01$) after 30 days of exposure to NaF and remained significantly ($p<0.01$) lower even on day 14 after termination of exposure to NaF. The ACP activity was also reduced significantly ($p<0.01$) on day 7 of exposure and did not return to the day 0 value even on day 7 after termination of exposure. The ALT activity decreased significantly ($p<0.01$) within 3 days of exposure. Its activity declined up to 46% after 30 days of F administration, and this activity did not return to pretreatment values even 14 days after termination of treatment. The AST activity increased in an exposure-dependent manner and increased 200% or 3- fold after 30 days of exposure. However, the enhancement of AST activity was seen only during F exposure, and it returned to pre-exposure values within 2 weeks after F

treatment. The plasma LDH activity did not show any significant changes from its pre-exposure activity throughout the period of administration of F.

Table 3. Effect of oral administration of NaF (20 mg/kg bw/day) in conjunction with treatment for 30 days by $Al_2(SO_4)_3 \cdot 16H_2O$ (150 mg/kg bw/day) on enzymatic parameters (mean ± SEM) (n=4)

Parameter	Treatment days							
	0	1	3	7	14	21	28	30
RBC AChE (nmol thiol group formed/ min/mL)	7307.6 ±747.9	6790.0 ±245.2	6788.0 ±285.8	5589.8 * ±123.81	7311.2 ±253.53	7914.3 ±501.36	6256.7 ±143.2	7133.9 [a*] ±432.38
Plasma AChE (nmol thiol group formed/ min/mL)	240.61 ±9.14	185.83 [†] ±5.64	215.05 ±11.31	131.33 [†] ±8.07	237.90 ±16.33	196.65 ±18.91	193.27 ±23.95	123.71 [a†] ±12.47
ALP (nmol phenol produced/ min/mL)	49.50 ±8.08	29.12 ±5.96	24.45 ±4.90	24.61 * ±2.37	18.68 [†] ±1.02	16.38 [†] ±0.73	12.74 [†] ±2.50	6.23 [a†] ±1.18
ACP (nmol phenol produced/ min/mL)	0.478 ±0.03	0.354 ±0.14	0.229 [†] ±0.04	0.784 [†] ±0.05	0.318 ±0.06	0.322 ±0.07	0.574 ±0.13	0.472 [a] ±0.24
ALT (nmol pyruvate formed/ min/mL)	12.83 ±1.49	13.48 ±0.76	9.53 ±1.04	3.84 [†] ±0.72	3.44 [†] ±0.73	6.87 * ±0.85	6.16* ±1.21	5.71 [a†] ±0.41
AST (nmol pyruvate formed/ min/mL)	31.90 ±5.28	36.89 ±3.27	24.67 ±2.48	31.43 ±2.45	19.06 ±3.36	20.37 ±1.36	16.02 [†] ±0.91	28.34 [a] ±1.02
LDH (nmol pyruvate reduced/ min/mL)	130.73 ±10.71	132.99 ±15.15	134.39 ±13.46	144.16 ±14.56	136.98 ±10.84	123.96 ±18.32	148.5 ±21.62	151.25 [a] ±16.39

[a] Mean of three animals.
*[†]Significantly different as compared to pre-exposure (day 0) value at 5% ($p < 0.05$) and 1% ($p < 0.01$), respectively.
AChE: Acetylcholinesterase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase; LDH: Lactate dehydrogenase; nmol: nano moles.

In the Group 2 goats, the erythrocyte AChE activity decreased during the first week of F exposure, but after that it did not show any significant variation. The plasma AChE activity was reduced significantly ($p<0.01$) on day 1 of exposure and remained lower during exposure. The ALP activity decreased significantly ($p<0.05$) on day 7 of exposure. Its activity remained significantly lower during F exposure and was at a minimum on day 30. The ACP activity showed a significant ($p<0.01$) decrease on day 3 but a significant ($p<0.01$) increase on day 7. After that, no significant pattern in ACP activity was observed. The ALT activity showed a significant ($p<0.01$) decrease on day 7 and remained significantly decreased during F exposure. There was a nonsignificant change in the activity of AST, except on day 28 of exposure. The LDH activity, however, did not show any significant changes throughout the period of F administration.

Table 4. Effect of oral administration of NaF (20 mg/kg bw/day) after treatment for 30 days by $Al_2(SO_4)_3 \cdot 16H_2O$ (150 mg/kg bw/day) on enzymatic parameters (mean ± SEM) (n=4)

Parameter	Post -treatment days		
	3	7	14
RBC AChE (nmol thiol group formed/min/mL)	4885.23 ± 259.06 [a*]	5433.44 ± 64.04 [a*]	7995.98 ± 286.80 [a*]
Plasma AChE (nmol thiol group formed/ min/mL)	128.04 ± 15.21 [a†]	154.91 ± 28.70 [a*]	207.56± 12.38 [a]
ALP (nmol phenol produced/ min/mL)	6.25 ± 1.76 [a†]	8.05 ± 1.79 [a†]	12.74 ± 2.69 [a†]
ACP (nmol phenol produced/min/mL)	0.611 ± 0.17 [a]	0.738 ± 0.17 [a]	0.870± 0.34 [a]
ALT(nmol pyruvate formed/min/mL)	7.41 ± 1.75 [a]	12.04 ± 0.37 [a]	5.45 ± 0.91 [a†]
AST (nmol pyruvate formed/min/mL)	25.61 ± 0.83 [a]	21.78 ± 3.17 [a]	21.45± 0.75 [a]
LDH (nmol pyruvate reduced/min/mL)	168.55 ± 32.8 [a]	173.1 ± 43.06 [a]	142.11± 23.62 [a]

[a] Mean of three animals.
*†Significantly different as compared to pre-exposure (day 0) value at 5% ($p < 0.05$) and 1% ($p < 0.01$), respectively.
AChE: Acetylcholinesterase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase; LDH: Lactate dehydrogenase; nmol: nano moles.

DISCUSSION

Acetylcholinesterase is an enzyme that has an important role in synaptic transmission. Its decreased activity leads to a diminished production of choline for the re-uptake process, thus decreasing the synthesis of new acetylcholine and in turn disturbing the synaptic transmission. The decreased activity of the enzyme in the present study may be responsible for excessive accumulation of acetylcholine in the synaptic cleft, and this excess acetylcholine can even cause neuromuscular paralysis throughout the entire body, leading to death by asphyxiation. F in high concentration is known to cause inhibition of the activity of the cholinesterase enzyme.[19] Paul et al.[20] observed decreased cholinesterase activity in the blood in rats. Chandra[21] has also reported inhibition of plasma cholinesterase enzyme activity during acute F toxicity of calves. Contrarily, however, Marconi found increased AChE activity in buffalo calves.[22]

With respect to ALP activity, its decrease in this study is consistent with the findings of Kessabi et al.,[23] who observed decreased ALP activity from acute NaF poisoning in sheep. The reduction in phosphatase activity may be due to formation of insoluble/inactive complexes with phosphates in acid and alkaline phosphatase.[24] Radostitis et al.[25] also observed a decreased level of ALP activity

during chronic fluorosis in sheep. Here it should be noted that F also inhibits enzymes required for bone and tooth formation. With decreased ALP activity there is a decrease in the production of phosphopyruvate, which is substrate for bone phosphatase.[19] Acute hepatitis and degeneration in the liver have also been reported in sheep following exposure to F.[26] In agreement with a number of earlier reports,[27-30] an increase in plasma AST activity was also observed during this study, which may be damaging to different tissues like the liver, heart, kidney, and muscle.

Any increase in levels of ALT and AST are of greater concern than reduced activities of these enzymes, since the levels of ALT activity are more specific indicators of liver damage than AST levels. From the present study, we can therefore suggest that the dose of sodium fluoride was not sufficient to produce liver damage in the goats. In their studies, Fidanci and Sel[29] observed a reduction in plasma ALT activity in sheep during summer and autumn.

The LDH levels also increased with damage to the liver. In the present study, negligible changes in LDH levels occurred, indicating no liver damage at this dose. Earlier findings indicated that LDH activity varies in fluorosis.[28,29,31] In the Group 2 goats, similar changes in enzymes, except AST, were observed. As reported earlier,[10,12] aluminium sulphate has not shown promising ameliorative effects in the antioxidant and biochemical parameters in these goats. Hence we conclude that NaF alone as well as in combination with aluminium sulphate produced significant adverse alterations in various enzymes of the body during subacute F intoxication, and concurrent administration of aluminium sulphate failed to show ameliorative action. Clearly, however, the various deviations in the dose response patterns of the different enzyme activities are worth further detailed investigation.

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