FLUORIDE AND ALUMINIUM INDUCED TOXICITY IN MICE EPIDIDYMIS AND ITS MITIGATION BY VITAMIN C

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SUMMARY: Treatment of adult male mice with sodium fluoride (NaF, 10 mg/kg bw/day) and aluminium chloride ($AlCl_3$, 200 mg/kg bw/day) simultaneously for 30 days caused marked histological changes in the epididymis accompanied by decreased levels of protein and sialic acid and also lowered activities of adenosine triphosphatase (ATPase) and succinate dehydrogenase (SDH) in caput and cauda epididymides with evident alteration of sperm maturation in mice. Withdrawal of NaF+$AlCl_3$ for 30 days led to significant recovery in all parameters except ATPase. Simultaneous treatment with NaF+$AlCl_3$+vitamin C, however, resulted in significant recovery in both regions of the epididymides. These results indicate that the toxic effects are similar in the two regions, but among the parameters studied, ATPase seems to be the most sensitive and would likely affect sperm metabolism and motility.

Keywords: Aluminium and epididymides; Adenosine triphosphatase; Fluoride and epididymides; Mice epididymis; Protein; Succinate dehydrogenase; Sialic acid.

INTRODUCTION

Fluoride occurs in the environment in water, soil, air, food, and vegetation with a significantly increased body burden, especially from industrial sources.[1,2] Aluminium is also present in water, soil, air, and food, some forms of which are "aluminium accumulators."[3] Fluoride is known to potentiate the toxicity of aluminium by promoting its absorption in the gastrointestinal tract of chickens and its accumulation in bone.[4] The combined effects of fluoride and aluminium on reproductive organs, especially the epididymis, a site of sperm maturation in the male, are not fully known. Therefore the present investigation was undertaken to study the toxic effects of NaF+$AlCl_3$ treatment for 30 days on caput and cauda epididymides of mice and the effects of subsequent withdrawal of treatment and recovery after NaF+$AlCl_3$+vitamin C ingestion for 30 days.

MATERIALS AND METHODS

Adult male mice (*Mus musculus*) of Swiss strain weighing between 20 and 30 g were procured and treated as reported in an earlier paper.[5] The doses used were based on the LD_{50} of fluoride in male mice (54.4 mg F/kg bw/day[6]) and of aluminium chloride (400 mg Al/kg bw/day[7,8]).

After the respective treatments, the animals were sacrificed by cervical dislocation, and caput and cauda epididymides were excised, blotted free of blood, and utilized for histology by haematoxylene and eosin (HE) staining for determination of several biochemical parameters. The levels of protein[9] and sialic acid,[10] and the activities of adenosine triphosphatase (ATPase) (E.C.3.6.1.3)[11] and succi-

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nate dehydrogenase (SDH) (E.C.1.3.99.1)[12] in caput and cauda epididymides of mice of Group I-IV were assayed by the methods cited.

The mice were divided into four groups each containing 10-12 animals and treated according to the following protocol:

Group	Treatment and dose (10–12 animals in each group)	Duration (days)	Day of autopsy
I	Control+distilled water (DW)	-	a
II	NaF–treated (10 mg/kg bw)+$AlCl_3$–treated (200 mg/kg bw)	30	31st
III	Same as in Group II then withdrawal for an additional 30 days	30+30	61st
IV	Same as in Group II+Vitamin C (15 mg/animal/day) for 30 days	30+30	61st

[a]Sacrificed along with treated mice.

Statistical analysis: For all biochemical parameters, a minimum of 5 or 6 replicates were assayed, and the data were statistically analysed by Student's t test.

RESULTS

Caput epididymis histology: The histology of caput epididymis of control mice showed compactly arranged tubules with broad pseudostratified epithelia lined with stereocilia and spermatozoa in the lumen (Figure 1).

The NaF+$AlCl_3$ treatment for 30 days caused clumping of stereocilia, pyknosis of epithelial cell nuclei, vacuolisation in the epithelium, and reduction in sperm density (Figure 2). Withdrawal of treatment for 30 days (Group III) revealed little recovery since clumped stereocilia and the absence of spermatozoa were still observed (Figure 3). Administration of vitamin C along with NaF and $AlCl_3$ (Group IV) showed almost normal histology of caput epididymis (Figure 4).

Cauda epididymis histology: The histology of cauda epididymis of control mice revealed large tubules with thin pseudostratified epithelium lined with stereocilia and dense sperm bundles in the lumen (Figures 5 and 6).The NaF+$AlCl_3$ treatment for 30 days (Group II) caused a decrease in stereocilia, disorganized epithelium cell debris in lumen, and reduction in sperm density (Figures 7 and 8). Withdrawal of NaF+$AlCl_3$ treatment (Group III) showed some recovery in sperm density but not in the epithelium (Figure 9). Administration of ascorbic acid along with NaF and $AlCl_3$ to mice for 30 days (Group IV) showed almost normal tubules, epithelium with distinct nuclei, and sperm bundles in their lumen (Figures 10 and 11).

Biochemical parameters: The levels of protein in caput and cauda epididymis of NaF+$AlCl_3$ treated mice (Group II) declined significantly ($p<0.001$) as compared to the control mice (Group I). Significant recovery occurred in both regions (caput, $p<0.01$; cauda, $p<0.02$) after withdrawal of treatment for 30 days in Group III as compared to Group II. However, combined NaF+$AlCl_3$+vitamin C treatment (Group IV) resulted in a highly significant ($p<0.001$) recovery (Table) as compared to Group II.

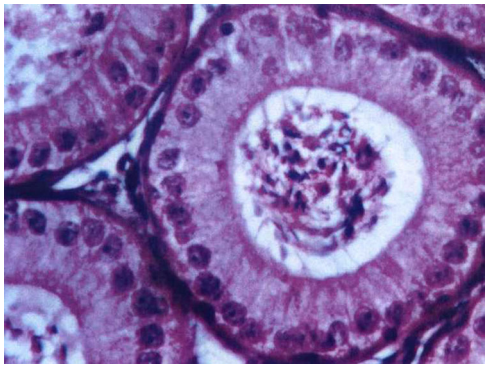

Figure 1. Transverse section of caput epididymis of a control Group I mouse. HE staining (X 850).

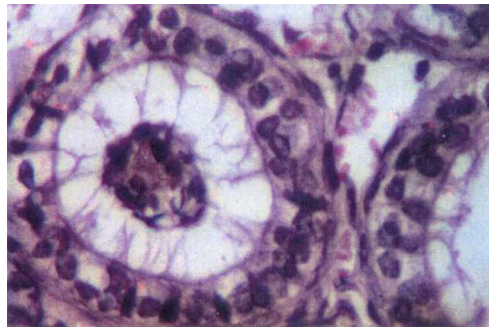

Figure 2. Transverse section of caput epididymis of a $NaF+AlCl_3$ (Group II) treated mouse. HE staining (X 860).

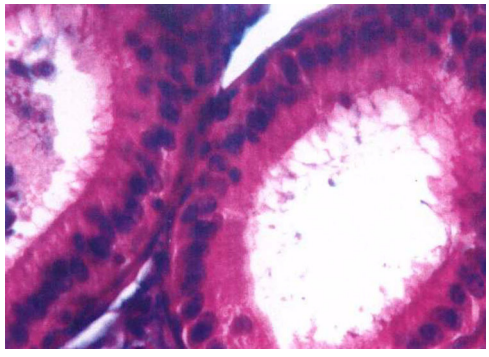

Figure 3. Transverse section of caput epididymis of a withdrawal (Group III) mouse. HE staining (X 880).

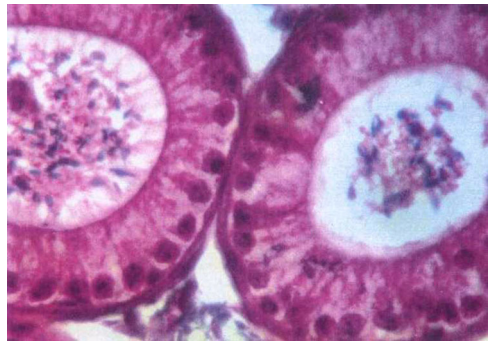

Figure 4. Transverse section of testis caput epididymis of a $NaF+AlCl_3$+vitamin C treated (Group IV) mouse. HE staining. (X 900).

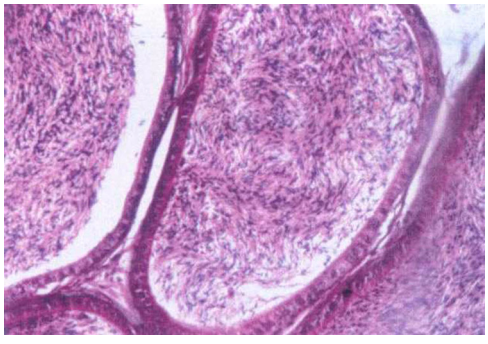

Figure 5. Transverse section of cauda epididymis of a control (Group I) mouse. HE staining (X 150).

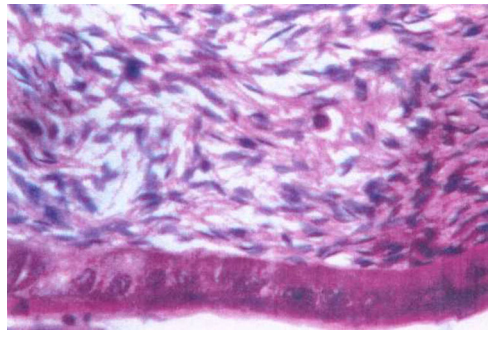

Figure 6. Magnified view of Figure 5. HE staining (X 870).

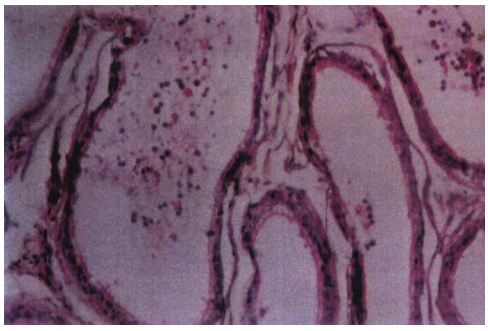

Figure 7. Transverse section of cauda epididymis of a NaF+$AlCl_3$ (Group II) treated mouse. HE staining (X 200).

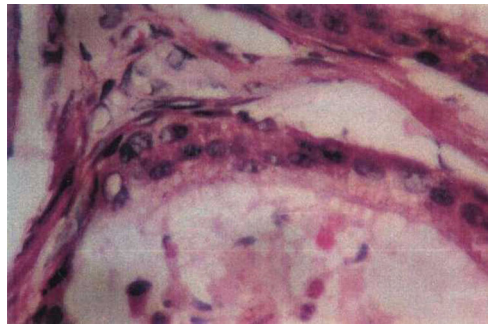

Figure 8. Magnified view of Figure 7. HE staining (X 600).

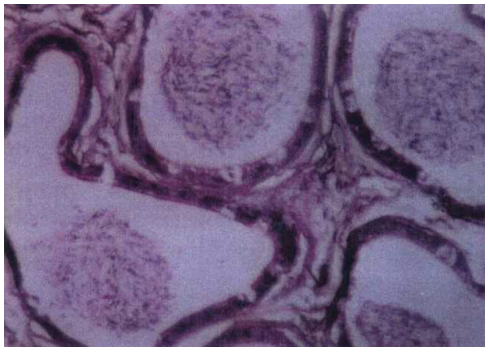

Figure 9. Transverse section of cauda epididymis of a withdrawal (Group III) mouse. HE staining (X 200).

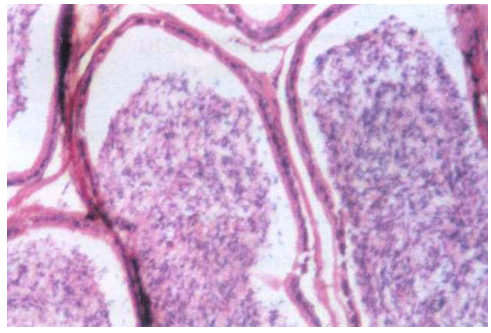

Figure 10. Transverse section of cauda epididymis of a NaF+$AlCl_3$+vitamin C (Group IV) treated mouse. HE staining (X 200).

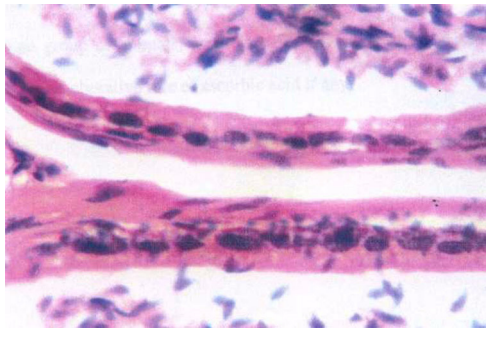

Figure 11. Magnified view of Figure 10. HE staining (X 740).

The levels of sialic acid and the activities of ATPase and SDH were also significantly decreased ($p<0.001$) in both caput and cauda epididymides in Group II mice as compared to the control mice. Recovery by withdrawal of treatment (Group III) in sialic acid and SDH ranged from $p<0.05$ to $p<0.01$ as compared to Group II. However ATPase activity was not recovered in both regions of epididymis (Table). All parameters were recovered significantly in Group IV by $NaF+AlCl_3$+vitamin C treatment as compared to Group II (Table).

DISCUSSION

In this study, treatment of mice with NaF and $AlCl_3$ for 30 days caused marked changes in the histology of both the caput and cauda epididymides. Disruption of epithelium with pycnotic cell nuclei, clumping of stereocilia, reduction in sperm density, and cell debris in the lumen were the major alterations as compared to controls. It is likely that these structural alterations would affect its epithelium and biochemical makeup and subsequently its internal milieu thereby making it nonconducive for sperm maturation and survival.[7,8,13-18]

Table. Protein (mg/100 mg fresh tissue wt), sialic acid (µg/mg fresh tissue wt) and activities of adenosine triphosphatase (ATPase) (µg ip released/mg protein/30 minutes) and succinate dehydrogenase (SDH) (µg formazan formed/mg protein/15 minutes) in caput and cauda epididymides of Groups I-IV mice[a]

Parameters	Organs	Group I	Group II	Group III	Group IV
Protein	caput epididymis	11.25 ± 0.16	6.70 ± 0.5§	8.14 ± 0.29‡	10.04 ± 0.25§
Protein	cauda epididymis	15.02 ± 0.05	5.72 ± 0.09§	7.64 ± 0.63†	12.04 ± 0.21§
Sialic acid	caput epididymis	4.39 ± 0.07	2.56 ± 0.14§	3.57 ± 0.28*	4.02 ± 0.09§
Sialic acid	cauda epididymis	5.74 ± 0.05	2.96 ± 0.64§	4.45 ± 0.02‡	5.44 ± 0.10•
ATPase	caput epididymis	2.11 ± 0.002	1.24 ± 0.09§	1.42 ± 0.06	2.02 ± 0.07§
ATPase	cauda epididymis	1.98 ± 0.04	0.88 ± 0.04§	1.06 ± 0.10	1.55 ± 0.04§
SDH	caput epididymis	13.70 ± 0.04	8.34 ± 0.77§	10.33 ± 0.38†	11.75 ± 0.23§
SDH	cauda epididymis	17.25 ± 0.03	9.21 ± 0.69§	11.42 ± 0.41†	15.06 ± 0.16§

[a]Data are expressed as mean ± SE; * $p<0.05$; † $p<0.02$; ‡ $p<0.01$; § $p<0.001$; where no sign = not significant
Comparisons: Group I with Group II; Group II with Group III; Group II with Group IV individually.

The protein levels decreased significantly in both caput and cauda epididymides in Group II mice as compared to control, which might inhibit the enzymes and secretions of the organ in agreement with earlier work.[13,14]

Sialic acid is essential for the maturation of spermatozoa in epididymis and maintenance of the structural integrity of their membranes. The levels of sialic acid in both regions of epididymides were decreased significantly. Hence the

structural integrity of the acrosomal membrane of the sperm might be affected causing loss of motility and subsequently infertility.[7,8,13,14,19]

The sperm adenosine triphosphatase (ATPase) plays an important role in supplying energy for sperm motility and metabolism. The restricted energy supply for the sperm as a result of significant decrease in ATPase will affect sperm motility as in the present study. Similar data in rats, mice, and rabbits have been reported earlier.[7,8,13,14,16]

The significant decline in epididymal succinate dehydrogenase (SDH) in $NaF+AlCl_3$ treated mice suggests a block in Krebs cycle in the conversion of succinate to fumarate. The mitochondrial structure and function are also likely to be disturbed, since SDH is a mitochondrial enzyme, and therefore oxidative metabolism will suffer.[7,8,13,14]

The withdrawal of the treatment (Group III) caused significant recovery in all parameters of caput and cauda epididymides except ATPase where recovery was insignificant. Hence, ATPase is especially sensitive to treatment as compared to other parameters.

The treatment with vitamin C along with $NaF+AlCl_3$, aided complete recovery of the epididymides. Vitamin C helps in recovery by virtue of its anti-oxidant properties and by increasing C-AMP levels[20] restoring enzyme activities, metabolism, and growth.

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