CHANGES IN TESTIS PROTEIN AND METABOLIC ENZYME ACTIVITIES IN RATS INDUCED BY SODIUM FLUORIDE AND SULFUR DIOXIDE

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SUMMARY: In experiments to assess the changes in testis protein and enzyme activities induced by exposure to sodium fluoride (NaF) and sulfur dioxide (SO_2), ninety-six adult male Wistar rats were divided randomly into four groups of twenty-four rats each. One group was left untreated as controls, and the other three groups were administered, respectively, for eight consecutive weeks, (1) 100 mg NaF/L (45 mg F^-/L) in their drinking water, (2) SO_2 in ambient air (15 ppm SO_2, 4 hr/day), or (3) were exposed to both NaF and SO_2 together. In comparison with the control group, the combination of NaF and SO_2 resulted in lower protein levels and higher lactate dehydrogenase (LDH) activities in the testis of male rats than in the groups treated by NaF or SO_2 alone. The activity of gamma-glutamyl transpeptidase (g-GT) decreased significantly in the NaF+SO_2 group and in the SO_2 group at week 4, and then increased markedly at weeks 6 and 8 in the same group. However, an increase in the activity of Na^+K^+- and Ca^{2+}-ATPases in the SO_2 group and the NaF+SO_2 group occurred at week 2 and then decreased at weeks 4 and 6, respectively. Mg^{2+}-ATPase activity at week 4 in the testis of male rats was significantly lower in all three treatment groups. These changes in protein and spermatogenesis-dependent enzymes undoubtedly affect the physiological functions of the testis, which may thereby cause low sperm motility.

Key words: ATPases; Enzyme activities; Fluoride and sulfur dioxide; Gamma-glutamyl transpeptidase; Male rats; Testis enzymes; Testis protein.

INTRODUCTION

Increasing attention to recent trends in declining reproductive health, especially the alarming decrease in male fertility rates worldwide, has led to the 21st century being dubbed the century of reproductive health.[1] In the light of such trends, the importance of studying the effects of environmental factors on male reproductive function is clear. It is known, for example, that high fluoride (F) levels adversely affect male reproduction function, and more than fifty publications since 1990 have focused on the reproductive effects of fluoride.[2] Moreover, in recent years, epidemiological investigations indicate that sulfur dioxide (SO_2) also affects male reproduction.[3] In fact, high concentrations of F and SO_2 are present in various environments and localities, and in some areas they coexist.[4-9] Thus, an investigation not only focusing on the effects of F or SO_2 exposure on male reproduction alone, but also measuring their interactive effects on the male reproductive system might prove helpful in determining potential causes of impaired reproductive functions in males.

Our recent investigations have revealed that the combination of F and SO_2 causes lower sperm motility in male rats[10] and that fluoride affects male reproduction through altering testis mass, cellular morphology, and normal spermatogenesis.[11-13]

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Protein and metabolic enzymes, such as lactate dehydrogenase (LDH) and adenosine triphosphatases (ATPases), are important for the normal function and development of the testis and for sperm motility.[14-17] Therefore, in the present study we investigated changes in total protein content and spermatogenesis-dependent enzymes of the testis in order to explore possible pathways of decreased sperm motility and altered levels of serum testosterone induced by NaF and SO_2 as we reported recently.[10]

MATERIALS AND METHODS

Experimental materials: Twelve-week old (mature) male Wistar albino rats, each weighing approximately 160 g, were obtained from the Experimental Animal Center of Shanxi Medical University together with their standard diets. The same pure SO_2 gas (99.99%) and the apparatus for NaF and SO_2 administration used in our previous studies[10,13] were employed here for this investigation.

Establishment of animal model: As in our recent reports,[10,13] ninety-six of the above male rats were randomly divided into four groups of twenty-four each: (1) a control group, which was given distilled water and clean air; (2) a NaF group, to which 100 mg NaF/L (= 45 mg F^-/L) was administered in their drinking water; (3) a SO_2 group, which was maintained continuously for 4 hr/day from 8:00 am to 12:00 noon every day each week in an ambient air concentration of 15 ppm SO_2; and (4) a NaF+SO_2 group to which the above treatments of both NaF and SO_2 together were administered. The F levels in the diet and drinking water and the SO_2 concentration in the ambient air are shown in Table1.

All rats were maintained on normal diets under standard temperature (22–25°C), 12/12-hr light/dark cycle, ventilation, and hygienic conditions. At the end of weeks 2, 4, 6, and 8, six rats from each group were randomly selected and sacrificed for further study.

Assay of protein and enzymes activities in testis: At day 14, 28, 42, and 56, the experimental rats were sacrificed by cervical dislocation, and the right testes were quickly collected, weighed, and then homogenized with 1:9 (w/v) 0.9% saline solution at 0–4°C. Total protein content and the activity of the gamma-glutamyl transpeptidase (γ-GT), lactate dehydrogenase (LDH), and ion-activated adenosine triphosphatases (ATPases) in testis tissues were determined with the enzyme reagent kit provided by the Nanjing Jianchen Biological Institute.

Table 1. Fluoride levels in diet (mg/kg) and drinking water (mg F^-/L) and sulfur dioxide (SO_2) concentration in ambient air (ppm)

	Control	NaF	SO_2	NaF+SO_2
Fluoride in diet	23.39±1.04	23.39±1.04	23.39±1.04	23.39±1.04
SO_2 in ambient air	<0.1[a]	<0.1[a]	15.0±5.0[b]	15.0±5.0[b]
Fluoride in drinking water	<0.6	45[c]	<0.6	45[c]

[a]Sulfur dioxide gas in air could not be detected below 0.1 ppm with our gas monitor.

[b]15 ppm sulfur dioxide emission was maintained continuously for four hours from 8:00 am to 12:00 noon every day each week; ±5.0 indicates the maximum (20 ppm) and minimum (10 ppm) SO_2 concentration extremes, not standard deviation or standard error.

[c]From 100 mg NaF/L.

RESULTS

Total protein content and activities of γ-GT, LDH, and ATPases in testis tissues of male rats are shown at Table 2, 3, 4 and 5, respectively.

Table 2. Testis total protein content (g/L) of male rats (n=6; mean±SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	0.71±0.03	0.59±0.03*	0.57±0.05*	0.56±0.03*
4	0.70±0.01	0.64±0.02	0.65±0.03	0.68±0.03
6	1.06±0.09	0.96±0.10	0.81±0.03*	0.75±0.02*
8	0.78±0.05	0.80±0.01	0.86±0.09	0.67±0.10
Mean value	0.81±0.04	0.74±0.08	0.69±0.04*	0.67±0.03*

*P<0.05 (compared with the control group).

Table 3. γ-GT activity (U/g protein) in testis of male rats(n=6; mean±SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	1.17±0.09	1.21±0.10	1.23±0.09	1.19±0.06
4	1.12±0.22	1.06±0.06	0.60±0.08†	0.66±0.08†
6	0.49±0.02	0.46±0.03	0.70±0.07†	0.63±0.04†
8	0.62±0.09	0.69±0.06	0.58±0.03	0.97±0.14*
Mean value	0.76±0.08	0.81±0.09	0.81±0.08	0.87±0.07

*P<0.05; †P<0.01 (compared with the control group).

Table 4. LDH activity (U/g protein) in testis of male rats (n=6; mean±SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	9.41±0.35	11.61±0.48*	11.45±0.67*	12.41±0.65†
4	9.47±0.32	10.79±0.50*	10.37±0.59	11.12±0.28*
6	6.57±0.07	7.90±0.87	10.32±0.47†	10.33±0.35†
8	10.27±0.35	10.58±0.38	9.94±0.89	14.27±1.67*
Mean value	8.93±0.35	10.19±0.42*	10.50±0.33†	11.21±0.28†

*P<0.05; †P<0.01 (compared with the control group).

Table 5. ATPases activity (μmol Pi/mg protein/hr) in testis of male rats (n=6; mean±SD)

	Treatment weeks	Control	NaF	SO_2	NaF+SO_2
Na^+K^+-ATPase	2	3.26±0.28	4.25±0.64	4.71±0.33*	4.61±0.09*
	4	3.93±0.49	3.31±0.34	2.83±0.28	3.14±0.13
	6	6.01±0.70	4.40±0.52*	4.19±0.11†	3.69±0.21†
	8	5.15±0.66	5.07±0.45	4.33±0.36	4.66±0.21
Ca^{2+}-ATPase	2	1.74±0.25	2.24±0.14	2.30±0.17*	2.44±0.13*
	4	2.50±0.23	1.84±0.21*	1.57±0.21†	1.79±0.19*
	6	3.27±0.47	2.74±0.39	2.77±0.28	2.48±0.23
	8	3.00±0.39	4.11±0.50	3.34±0.70	4.06±0.11
Mg^{2+}-ATPase	2	1.88±0.33	2.05±0.21	2.24±0.16	2.16±0.11
	4	2.14±0.19	1.53±0.19*	1.36±0.16†	1.58±0.09*
	6	4.11±0.58	3.63±0.50	3.99±0.32	3.23±0.25
	8	3.89±0.41	5.05±0.59	4.32±0.70	4.75±0.28

*P<0.05; †P<0.01 (compared with the control group).

DISCUSSION

Effect of NaF and SO_2 on testis protein metabolism of male rats: Earlier studies have reported a dose-dependent decrease in protein levels in the spermatozoa and reproductive organs of fluoride-treated animals.[2,14,17-20] In this study, the total protein levels in the testis of male rats decreased significantly in the NaF group at week 2 and in the SO_2 and NaF+SO_2 groups at weeks 2 and 6 compared with the control group. The mean total protein content over the entire 8-week period was slightly lower in the NaF group but was drastically decreased in the SO_2 and NaF+SO_2 groups. These results clearly suggest that the combination of NaF and SO_2 leads to lower testis protein content than either F or SO_2 alone.

Gamma-glutamyltranspeptidase (Gamma-glutamyltransferase, γ-GT), is the key enzyme in the γ-glutamyl cycle, and plays an important role in the absorption, transport, and synthesis of amino acids and proteins.[21] Zakzrewska et al.[17] suggested that the activity of γ-GT decreased significantly in ram semen with ingestion of 20 to 200 μmol NaF/L, but returned to initial levels with 0.1mol NaF/L. Increased γ-GT activities occurred in the hemolymph of F-poisoned silkworm larvae[22] and in the serum of male rats ingesting 20 mg NaF/kg bw for six months.[23] In the present study, γ-GT activity in the NaF+SO_2 group decreased significantly at week 4 and then increased markedly at weeks 6 and 8 compared with the control group values. The same trend was found in the SO_2 group. Obviously, changes in γ-GT activity induced by F are complicated and require further study. Nevertheless, it can be concluded that both protein metabolism and synthesis in testis of male rats is affected by NaF and SO_2 or their combination, and the influence of F or SO_2 on the γ-glutamyl cycle may be responsible for a reduction in testis protein of male rats. The reduction in testis proteins may also be due to interference by F or sulfite (in the form of metabolites of SO_2 *in vivo*) with binding of the amino acyl-t-RNA adducts to the ribosomal RNA template, which would then inhibit biosynthesis of protein in testis.[14]

Effect of NaF and SO_2 on testis metabolic enzyme activities of male rats: Lactate dehydrogenase (LDH) and ion-activated adenosine triphosphatases (ATPases) are important for spermatogenesis and testicular metabolism.[15,16] Many studies have indicated that the activity of these enzymes decreases in testes of F-treated animals.[2,14,17] In our study, LDH activities increased in the NaF group at weeks 2 and 4, in the SO_2 group at weeks 2 and 6, and in the NaF+SO_2 group from weeks 2 to 8. These results are in accord with those obtained by Zakrzewska et al.[17] when they went from lower to higher concentrations of NaF in treating ram sperm samples. Furthermore, the mean values for the entire 8-week period were significantly increased in the three treatment groups compared to the controls. These findings indicate that the interaction of NaF and SO_2 can result in higher LDH activity than either SO_2 or NaF alone.

On the other hand, our observations over time show that the activities of Na^+K^+-ATPase at week 6, Ca^{2+}-ATPase, and Mg^{2+}-ATPase at week 4 in the three treatment groups all decreased significantly as compared with the controls, whereas the activities of Na^+K^+- and Ca^{2+}-ATPase increased at week 2 in the SO_2

and NaF+SO_2 groups. Evidently, the effect of F and SO_2 on ATPase activities is not straightforward.

It has been reported that the toxic effect of F on reproduction is due to the inhibition of many enzymes, particularly those whose cofactor is the cation of a bivalent metal.[17] It is likely, therefore, that some of the deceased enzyme activity found in the present study is the result of damaged testis tissue. However, others have reported that a lower dose F can increase enzymes activity by a positive feedback mechanism.[24] Alternatively, the changes in testis metabolic enzyme activities of male rats may be related to both dose and duration of F intoxication. Likewise, sulfate and sulfite, which are both *in vivo* metabolic products of SO_2 inhalation, can inhibit or activate activity of certain enzymes by inducing the production of free radicals.[25] In any case, it is evident that with exposure to both F and SO_2 various types of lesions were likely to result.

It should be noted, however, that LDH, which is regarded as a marker of carbohydrate metabolism of germ-cell production and differentiation,[15] plays a key role in the process of energy supplementation for spermatozoa motility.[17] ATPases, including Na^+K^+-, Ca^{2+}-, and Mg^{2+}-ATPase, play a key role in exchange of metabolites between Sertoli and developing germ cells and are markers of the metabolic state of germinal epithelium.[16] The changes in these enzymes will undoubtedly affect physiological function in the testis, which may cause significant decline in sperm motility. Nevertheless, the exact alterations in testis physiological function caused by F and SO_2 are not clear, and histological and ultra-structural studies are called for in order to illuminate this aspect.

In conclusion, both F and SO_2 treatment alter testis protein and the activity of some enzymes in male rats, and these changes may be one of the pathways that lead to low sperm motility.

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