EFFECTS OF SODIUM FLUORIDE AND SULFUR DIOXIDE ON SPERM MOTILITY AND SERUM TESTOSTERONE IN MALE RATS

Jianhai Zhang,[a] Chen Liang,[a] Junjie Ma,[a] Ruiyan Niu,[a] Jundong Wang[a]
Shanxi, China

SUMMARY: Ninety-six sexually mature male Wistar rats were divided randomly into four groups of twenty-four rats. In experiments to assess effects of sodium fluoride and sulfur dioxide on their sperm motility and serum testosterone (T), one group of rats was left untreated as controls, and the other three groups were administered, respectively, for eight consecutive weeks, 100 mg NaF/L (45 mg F^-/L) in their drinking water, sulfur dioxide in ambient air (15 ppm SO_2, 4 hr/day), or were exposed to both NaF and SO_2 together. In comparison with the control group, the ratio of testis weight to body weight increased in the NaF+SO_2 group at week 4, and the mean values of sperm motility were significantly lower in the NaF group and in the SO_2 group, and even more so in the NaF+SO_2 group over the entire eight-week period. The serum T level in the NaF+SO_2 group was significantly increased at week 2 and then markedly decreased at week 4 and 8. Similar changes were also observed in the SO_2 group. Sperm motility and serum T in rats were thus affected to some extent by NaF or SO_2 but were most drastically affected by NaF and SO_2 together. The resulting changes in the testis tissue and serum T concentration may therefore be connected with the low sperm motility in these rats.

Keywords: Fluoride and sulfur dioxide; Male rats; Reproductive hormones; Sperm motility.

INTRODUCTION

Over the past fifty years the mean sperm count and sperm volume in healthy men are reported to have declined by 50% worldwide,[1] a decrease that can be correlated with increases in environmental pollutants.[2] Consequently, concern about effects of environmental changes on male reproductive health has become a major preoccupation in many countries.[2]

Fluorides and sulfur dioxide (SO_2) are two well-known toxic pollutants to which humans are exposed. [3] In recent years, the adverse effects of sodium fluoride on the male reproductive system have been studied by our research group.[4-6] Moreover, other recent investigations indicate that fluoride (F) and SO_2 emissions co-exist in some areas, such as in coal-burning fluorosis areas,[7-9] aluminum smelter workplaces,[10] and volcanic fog areas.[11,12] On the other hand, a decline in the semen quality of young Czech men exposed to seasonal air pollution consisting primarily of SO_2 has been reported,[13] but there appear to be few reports indicating a direct correlation between high SO_2 levels and male reproductive dysfunction. The aim of this investigation, therefore, is to evaluate through an animal study the relationships that might exist between impaired reproductive function and exposure to F, SO_2, or the two combined.

MATERIALS AND METHODS

Materials: Twelve-week-old (mature) male Wister albino rats (each weighing approximately 160 g), along with supplies of their standard diet, were obtained from the Experimental Animal Center of Shanxi Medical University. Pure sulfur

[a]For Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, P.R. of China. E-mail: wangjd@sxau.edu.cn

dioxide gas (SO_2) (99.99%) was provided by Foshan Kedi Gas Chemical Industry Co., Ltd, Guangdong, China.

Establishment of animal model: Ninety-six of the above male rats were randomly divided into four groups of twenty-four: a control group, a sodium fluoride (NaF) group, a sulfur dioxide (SO_2) group, and a sodium fluoride plus sulfur dioxide (NaF+SO_2) group. The F levels in their diet and drinking water and the SO_2 concentration in the ambient air are shown in Table 1. All rats were maintained on the standard diets under normal conditions of temperature (22–25°C), 12/12-hr light/dark cycle, ventilation, and good hygiene.

Table 1. Fluoride levels in diet (mg F^-/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 during every exposure day; ±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.

At the end of weeks 2, 4, 6, and 8, six rats from each group were randomly selected, weighed, and injected with 20% urethane solution for lethal anesthesia. Blood was collected from the eyeball for separating serum, and the testes and epididymides tissues were carefully removed and blotted free of blood for further study. Urinary F levels were determined using an ion selective electrode. Lung tissues of rats exposed to SO_2 and NaF+SO_2 were fixed and prepared for histopathological examination. In addition, the body weights of the surviving rats were checked weekly.

Experimental design for SO_2 exposure treatment groups: For exposure to SO_2 the rats were housed in a wood panel cabinet-type smokehouse with glass windows measuring 1.5×0.7×1.0 m. Four circulating fans provided air exchange, and a plastic tube by which SO_2 was delivered and dispersed evenly in the chamber. The concentration of SO_2 in the ambient air was monitored continuously with a PGM-35 unit obtained from RAE Systems Inc., USA.

Evaluation of sperm motility in male rats: The right epididymal sperm suspension was prepared at 37°C in normal saline; the spermatozoa motility was measured according to the method of Cui et al.[14]

Assays of blood serum testosterone*:* The concentrations of testosterone (T) in the experimental rat serum samples were determined by RIA (radioimmunoassay) with reagent kits provided by the Chinese Institute of Atomic Energy, Beijing, China.

RESULTS

Animal model: Over the whole experimental period, the mean urinary F levels in the control group and three treatment groups were 8.37±3.19 mg/L, 40.53±4.22 mg/L, 14.11±3.87 mg/L, and 42.56±4.04 mg/L, respectively. Meanwhile,

histopathological changes in the lungs were observed in both the SO_2 group and the NaF+SO_2 group. Thus the animal model was satisfactorily established.

Development of male rats: The body weight records and the ratio of the testis weight to body weight over the period of treatment in all groups are shown in Tables 2 and 3, respectively.

Table 2. Eight-week record of body weight (g) of male rats (mean ± SD)

Treatment weeks	N	Control	NaF	SO_2	NaF+SO_2
1	24	172.05±17.37	169.41±12.87	171.89±13.57	169.19±14.51
2	24	285.30±14.70	274.86±7.08	270.70±3.63*	268.16±7.00*
3	18	277.55±10.49	271.88±6.68	253.53±9.48	256.60±8.97
4	18	285.74±21.53	277.00±7.96	246.64±11.81	225.40±9.13*
5	12	304.62±12.28	297.56±10.59	268.23±9.32	260.25±14.13*
6	12	315.88±20.45	291.18±13.90	280.07±5.73	280.12±10.55
7	6	312.96±15.90	324.36±18.15	285.87±11.12	287.24±11.30
8	6	311.38±17.12	337.03±10.27	279.73±16.36	262.62±39.33*

*p<0.05 compared with the control group.

Table 3. Percentage of testis weight to body weight (g/g) in male rats (n=6; mean ± SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	0.91±0.02	0.94±0.05	0.98±0.03	0.96±0.03
4	0.95±0.03	1.00±0.04	1.08±0.05	1.17±0.07†
6	0.90±0.07	0.93±0.02	0.96±0.03	0.99±0.03
8	0.86±0.05	0.78±0.03	0.96±0.06	0.88±0.03
Mean value	0.906±0.013	0.914±0.024	0.998±0.019*	1.002±0.029*

*p<0.05, †p<0.01 compared with the control group.

Sperm motility of male rats: The percentages of the sperm motility in each group are recorded in Table 4.

Table 4. Sperm motility percentage in male rats (n=6; mean ± SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	86.70±3.16	78.06±3.54	73.82±3.72*	49.71±2.97†
4	64.73±3.33	58.09±1.20	52.87±5.73*	55.89±0.85
6	59.52±4.11	41.63±3.45†	50.36±6.13	40.47±4.11†
8	71.28±2.18	64.11±2.56	65.11±1.87	58.22±2.64†
Mean value	70.56±3.41	60.47±3.27*	60.13±3.37*	51.07±2.19†

*p<0.05, †p<0.01 compared with the control group.

Serum testosterone level: The concentrations of testosterone (T) in the serum of experimental rats are shown in Table 5.

Table 5. Testosterone (T) concentration (ng/dL) in male rat serum (n=6; mean ± SD)

Treatment weeks	Control	NaF	SO_2	NaF+SO_2
2	390.39±41.27	338.12±50.04	729.61±186.30	917.46±247.61*
4	378.81±23.54	412.17±23.12	219.09±45.47*	182.15±62.44†
6	468.43±151.34	331.50±64.18	501.17±142.19	372.22±65.69
8	310.64±99.17	396.42±42.35	153.68±27.56*	155.28±39.62*

*p<0.05, †p<0.01 compared with the control group.

DISCUSSION

Effects of NaF and SO_2 on growth and development: In this study, the rate of body weight increase of the rats decreased significantly in the NaF+SO_2 group at weeks 2, 4, 5, and 8 and in the SO_2 group at week 2 after treatment. Likewise, the ratio of testis weight to body weight also increased significantly in the NaF+SO_2 group at week 4, and the mean values of this ratio were significantly higher over the entire 8-week period in the SO_2 as well as the NaF+SO_2 group. The latter results suggest that sexual development of male rats, especially in testicular tissue, was affected by the SO_2 treatment and by the combined treatment with NaF and SO_2.

Effects of NaF and SO_2 on sperm motility: Previous reports have shown that sperm motility is reduced in NaF-treated experimental animals,[6,15,16] and in areas of human endemic fluorosis.[17] However, some experimental results suggest that sperm motility is not affected by F.[18] In our study, sperm motility in the cauda epididyma of male rats was decreased significantly in the NaF group at week 6 compared to the controls, in the NaF+SO_2 group at weeks 2, 6, and 8, and also in the SO_2 group at weeks 2 and 4. The mean values of the entire 8-week period were significantly lower in the three treatment groups compared with the control group value. These findings indicate that the interaction of NaF and SO_2 can result in significantly lower sperm motility than either SO_2 or NaF alone.

Effect of NaF and SO_2 on serum testosterone: Past reports indicate that the change in serum testosterone (T) at a given time during F exposure is complicated. Chinoy suggested that the circulating serum T levels were only slightly changed in a human population of endemic fluorosis areas in India,[17] and an unchanged serum T concentration occurred in male workers exposed to F for twelve continuous years.[19] Other reports indicate that serum T levels decrease in skeletal fluorosis patients[20] and in experimental animals treated with F.[21,22] In the present study, however, our dynamic determinations showed that serum T levels did not change significantly in the NaF group during the entire 8-week period as compared with the control group. These differences in findings might be due to variations in dose, mode of delivery, and duration of F administration. Differences in the sensitivity of different animal species to F may also play an important role in these changes of serum T levels.

On the other hand, in this study, the changes in T level occurred at various stages in the male rat exposed to SO_2 alone or in combination with NaF. Thus the serum T levels in the NaF+SO_2 group were significantly increased at week 2 and then markedly lower by week 4, subsequently rising near to the control level at week 6 before notably falling again at week 8. Similarly, a decreased serum T level was observed in the SO_2 group at weeks 4 and 8. These results indicate that SO_2 inhalation and its combination with NaF ingestion can affect the production and secretion of T in male rats.

Possible mechanisms of how NaF and SO_2 affect male reproductive function: Sperm motility is one of the important indexes in evaluating male reproductive function,[22] and is closely associated with impaired spermatogenesis, which can cause a decline in sperm motility.[16] We postulate, therefore, that spermatogenesis

may be affected in the testis of male rats administered F and SO_2. On the other hand, normal spermatogenesis is not only related to the structure and function of the testis but is also regulated by the hypothalamic-pituitary-testicular axis (HPTA). T, which is produced by Leydig cell in testis, plays an important role in this regulation process. [24] Apparently, each checkpoint response induced by F and SO_2 can interfere with spermatogenesis and can disturb normal T levels, thereby reducing sperm motility.

In the present study, therefore, an increase of serum T in the NaF+SO_2 group at week 2 may be the result of compensatory and regulatory responses after treatment. The changes in serum T level and the testicular tissue weight in the same group also suggest that the normal structure and function in testis, especially in the Leydig cells, may be damaged by the interaction of F and SO_2 or SO_2 alone. Even so, the reason for the low sperm motility of male rats after ingestion NaF in the absence of SO_2 is still uncertain and clearly deserves further study.

CONCLUSION

Under the experimental conditions detailed above, sperm motility and the production and emission of testosterone in male rats were adversely affected by exposure to F and SO_2. The changes in the testis tissue and in serum testosterone may be one of the pathways that lead to low sperm motility of male rats.

ACKNOWLEDGEMENT

This research was sponsored by Shanxi Province Returnee's Science Foundation of China (Grant No. 2004043).

REFERENCES

1 Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. Br Med J 1992;305:609-13.
2 Zhang Y. Environmental pollution threatens human fertility. Environ Rev 2003;22:20-5. [in Chinese].
3 Cape JN, Fowler D, Davison A. Ecological effects of sulfur dioxide, fluorides, and minor air pollutants: recent trends and research needs. Environ Inter 2003;29:201–11.
4 Wan SX, Zhang JH, Wang JD. Effects of high fluoride on sperm quality and testicular histology in male rats. Fluoride 2006;39(1):17-21.
5 Wan SX, Zhang JH, Wang JD. Fluoride-induced changes in the expression of epidermal growth factor and its receptor in testicular tissues of young male rats. Fluoride 2006;39(2):121-5.
6 Zhang JH, Wang JD, Pang QH, Lang ZX. Effects of high fluoride on reproduction function of the experimental animals and humans. Proceedings of the 2nd National Conference on the History of Toxicology and Control of Paroxysmal Poisoning; 2004 June; Xi'an, China.Shaanxi; 2004. p. 20-4. [in Chinese].
7 Ge S, Xu X, Chow JC, Watson J, Sheng Q, Liu W, Bai Z, Zhu T, Zhang J. Emissions of air pollutants from household stoves: honeycomb coal versus coal cake. Environ Sci Technol 2004;38:4612-8.
8 Lin MQ, He LL, Xiao YD, Min D, Liu YQ, Chen YX. Relationship between total amount of fluoride intake and occurrence of dental fluorosis in coal-burning polluting-type areas of fluorosis. Chin J Endemiol 1996;15:197-9. [in Chinese].
9 Lang CK, He ZL, Cao HG, Min D, Li YP, Zhou M. Effects and simulation test of sulfur dioxide on coal-burning polluting-type fluorosis. Health Res 1993;22:148-51. [in Chinese].
10 Carter SR, Seixas NS, Thompson ML, Yost MG. Peak exposures in aluminium potrooms: instrument development and field calibration. J Environ Monit 2004;6:932-7.
11 Soyseth V, Kongerud J, Haarr D, Strand O, Bolle R, Boe J. Relation of exposure to airway irritants in infancy to prevalence of bronchial hyper-responsiveness in school children. Lancet 1995;345:217-20.

12 Delmelle P, Delfosse T. Sulfate, chloride and fluoride retention in Andosols exposed to volcanic acid emissions. Environ Pollut 2003;126:445-57.
13 Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP, Perreault SD. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Persp 2000;108:887-94.
14 Cui LX, Jiang CX, Wang XL, Chen XM. Experimental study on effect of fluoride on reproductive system of male rats. Chin J Endemiol 2003;22:195-7. [in Chinese].
15 Zakrzewska H, Udala J, Blaszczyk B. In vitro influence of sodium fluoride on ram semen quality and enzyme activities. Fluoride 2002;35:153-60.
16 Chinoy NJ, Sharma A. Reversal of fluoride-induced alteration in cauda epididymal spermatozoa and fertility impairment in male mice. Environ Physiol Toxicol 2000; 7:29-38.
17 Chinoy NJ. Studies on effects of fluoride in 36 villages of Mehsana district, North Gujarat. Fluoride 1992;25:101-10.
18 Collins TFX, Sprando RL, Black TN, Shackelford ME, Bryant MA, Olejnik N, Ames MJ, Rorie JI, Ruggles DI. Multigenerational evaluation of sodium fluoride in rats. Food Chem Toxicol. 2001;39:601-13.
19 Zhang XZ, Luo W, Xie YF. Study on male reproductive-incretion function affected by fluoride. Occup Med 1995;22:10-1. [in Chinese].
20 Susheela AK, Jethanandani P. Circulating testosterone levels in skeletal fluorosis patients, J Toxicol Clin Toxicol 1996;34:183-9.
21 Narayana MV, Chinoy NJ. Effect of fluoride on rat testicular steroidogenesis. Fluoride 1994;27:7-12.
22 Zhao ZL, Wu NP, Gao WH. Influence of fluoride on contents of testosterone and cholesterol in rat. Fluoride 1995;28:128-30.
23 Ortiz-Perez D, Rodriguez-Martinez M, Martinez F, Borja-Aburto VH, Castelo J, Grimaldo JI et al. Fluoride-induced disruption of reproductive hormones in men. Environ Res 2003;93:20-3.
24 Holstein AF, Schulze W, Davidoff MS. Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol 2003;1:107.
Available from: http://www.rbej.com/content/1/1/107