FLUORIDE-INDUCED CHANGES IN THE EXPRESSION OF EPIDERMAL GROWTH FACTOR AND ITS RECEPTOR IN TESTICULAR TISSUES OF YOUNG MALE RATS

Shuangxiu Wan,[a] Jianhai Zhang,[b] Jundong Wang.[b]

Shanxi, China

Summary: Sixty-four forty-day old male Wistar rats were randomly divided into two groups of thirty-two each. One group was left untreated as controls, and the other group was administered 150 mg NaF/L (68 ppm F^-) in their drinking water to assess changes in epidermal growth factor (EGF) and its receptor (EGFR) expression induced by fluoride in the testes by using an immunohistochemical assay. After ten consecutive days of exposure to fluoride, decreased EGF expression occurred in the Leydig cells, spermatogonia, and spermatocytes, along with diminished EGFR expression in the spermatocytes, and spermatids of testicular tissues. This decrease in expression of EGF and its receptor in Leydig cells and spermatogenic cells may be one of the pathways that can impair reproductive function.

Keywords: Epidermal growth factor (EGF); Epidermal growth factor receptor (EGFR); Fluoride and testes; Leydig cells; Male rats; Spermatocytes; Spermatogonia.

INTRODUCTION

Recent reports of adverse effects of fluoride on the male reproductive system have attracted considerable interest.[1-3] Clinical investigations and animal experiments suggest that fluoride can cause the impairment of reproductive function.[4-9] Our recent research indicated that 68 mg fluoride ion/L in the drinking water significantly reduces sperm quality and alters testicular histological parameters in male rats.[10] The epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) play an important role in spermatogenesis and testicular steroidogenesis.[11-13] However, there appear to be few reports that indicate some relationship between fluoride and EGF and EGFR expression in testicular tissues. This study, therefore, aims to explore changes in EGF and EGFR expression induced by fluoride in young rat testes.

MATERIAL AND METHODS

Materials: Forty-day-old male Wistar rats (each weighing approximately 50 g) and their standard diets were obtained from the experimental animal center of Shanxi Medical University for this study.

Establishment of animal model: As in our recent report,[10] sixty-four of the above male rats were divided randomly into two groups of thirty-two: a control group, which was given distilled water, and a high fluoride group to which 150 mg NaF/L (= 68 mg F^-/L) was administered in their drinking water. All rats were maintained on the standard diets at 22–25ºC under normal ventilation and hygienic conditions. At day 50 (i.e., at 50 days of age), eight rats from each group were randomly selected, weighed, and injected with 20% urethane solution for fatal anaesthesia. Blood was collected from the eyeball for separating serum, and the testes tissues

[a]Shandong Heze College, Heze, Shandong, 274015, PR of China.
[b]For Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, PR of China; E-mail: wangjd@sxau.edu.cn

were carefully removed, blotted free of blood, fixed in Karnory substrate,[14] and embedded by paraffin for further study.

Immunohistochemistry for EGF and EGFR: Fixed and paraffin-embedded testis sections six-micrometers thick of 50-day old rats were stained with EGF & EGFR immunohistochemistry kits, which were provided by Wuhan Boster Bioengineering Co., Ltd, Wuhan, China, by the method of Yang et al.[15] After deparaffination, the endogenous peroxidases of these sections were blocked with 0.3% H_2O_2, and forced reaction with EGF and EGF-R antibody (diluted by 1:100) for 24 hr at 4ºC. Similarly, instead of using EGF and EGFR antibody, normal rat serum was used for the negative control section. Subsequently, incubation with 1% rabbit anti-human serum, antigen retrieval was performed in a microwave oven for 20 min at 80ºC for 10 min. In a final step, sections were counterstained with hematoxylin and mounted under cover slips with neutral gum prior to observation using an upright microscope (model: BX51, OLYMPUS of Japan); 5–10 visual fields per slide, and 6 sections per male rats were selected randomly for analysis.[16] Cells that contained yellow or brown yellow granules were regarded as the positive cells, whereas the cells with no yellow or brown yellow granules were considered to be negative cells. Optical densities of the positive cells from every viewing were measured by Image-Pro® Plus Version 5.1 micrograph analysis software (made in Media Cybernetics Inc. of America).

RESULTS

Results of EGF expression in the testes of the 50-day-old male rats are shown in Figures 1 and 2 and in Table 1.

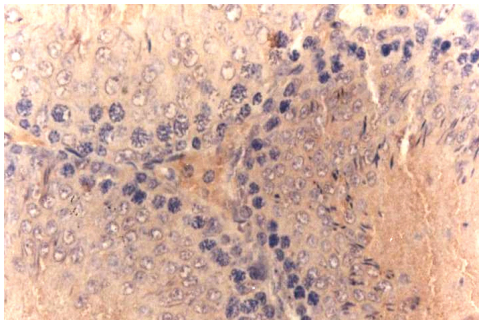

Figure 1. EGF expression in the testes of 50-day-old fluoride-exposed male rats (×660).

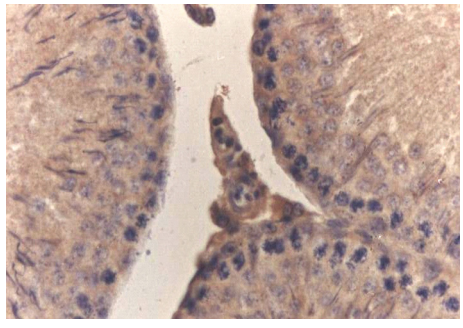

Figure 2. EGF expression in the testes of 50-day-old control male rats (×660).

Table 1. EGF expression measured as optical density in testicular tissue of 50-day-old male rats (mean±SE)

Expression cells or sites	Control	Fluoride-exposed
Leydig cells	0.3389±0.0053	0.3165±0.0022*
Spermatogonia	0.2528±0.0071	0.2193±0.0017*
Spermatocytes	0.2329±0.0045	0.2087±0.0026*
Spermatids	0.1896±0.0026	0.1910±0.0012
Lumen Sheddings	0.1468±0.0025	0.1447±0.0029

*p<0.01compared with the control group.

Changes in EGFR expression in the testes of the 50-day-old male rats are recorded in Figures 3 and 4 and in Table 2.

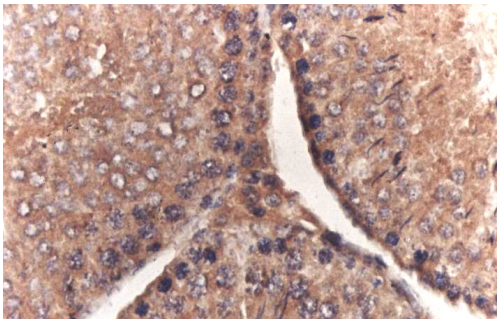

Figure 3. EGFR expression in the testes of 50-day-old fluoride-exposed male rats (×660).

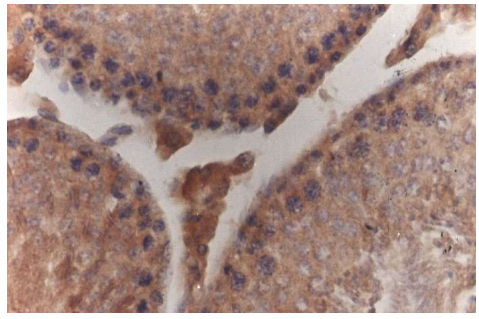

Figure 4. EGFR expression in the testes of 50-day-old control male rats (×660).

Table 2. EGFR expression measured as optical density in testicular tissue of 50-day-old male rats (mean±SE)

Expression cells or sites	Control	Fluoride-exposed
Leydig cells	0.3260±0.0014	0.3214±0.0009
Spermatogonia	0.3077±0.0040	0.3075±0.0019
Spermatocytes	0.3036±0.0022	0.2987±0.0009*
Spermatids	0.3029±0.0014	0.2968±0.0013†
Lumen sheddings	0.0397±0.0003	0.0400±0.0004

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

Views of typical negative controls, considered to lack EGF and EGFR expression in the testes, are shown in Figures 5 and 6, respectively.

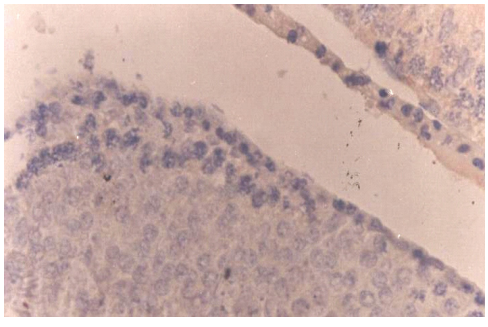

Figure 5. Example of negative control lacking EGF expression in the testes of 50-day-old male rats (×660).

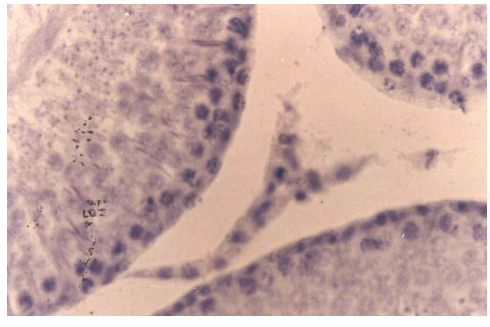

Figure 6. Example of negative control lacking EGFR expression in the testes of 50-day-old male rats (×660).

DISCUSSION

Epidermal growth factor is an androgen regulated mitogenetic growth factor that has been shown to regulate ectodermal and mesodermal cellular development.[17] The major source of EGF is from the submandibular gland and is increased by androgenic stimulation.[18] Tsutsumi et al.[11] and Leng et al.[19] reported that sialoadenectomy (removal of the submandibular glands) inhibited

spermatogenesis processes and testicular development in rats, and decreased the amount of circulating EGF to an undetectable level in mice. They also found that the number of mature sperm in the epididymis decreased by as much as 55 percent; and the number of spermatids in the testis decreased by 40 to 50 percent. Administration of EGF to sialoadenectomized mice restored both the sperm content of the epididymis and the number of spermatids in the testis to normal levels.[11] Thus, EGF may play a role in male reproductive function by stimulating the meiotic phase of spermatogenesis.

In the present study, compared to the controls, the expression of EGF in the testes of male rats drinking 68 ppm F^- water for 10 days showed a significant decrease in Leydig cells, spermatogonia, and spermatocytes. The EGFR expression also decreased in the spermatocytes and spermatids in the testes of these rats. Furthermore, as we have shown recently, sperm quality, the ratio of testicular weight to body weight, and testicular histological parameters were also altered in male rats under the same conditions.[10]

It should be noted that EGF apparently exerts its effects by binding to and activating EGFR. After binding to EGF ligands, EGFR triggers subcellular protein tyrosine phosphorylation, including rapid receptor autophosphorylation, which plays an important role in spermatogenesis.[20] Therefore, although the changes in EGF or EGFR expression only occur in some kinds of cells of testicular tissues, the combined effects of these changes may involve Leydig cells and all spermatogenic cells. At present the mechanism of the decrease of EGF and EGFR expression in the testis induced by fluoride is not clearly known.

In conclusion, fluoride decreased the EGF and EGFR expression in Leydig cells and spermatogenic cells of young male rat testes, which may be one of the pathways that lead to impaired reproductive function.

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