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Long-term effects of cigarette-smoke exposure on plasma testosterone, luteinizing hormone and follicle-stimulating hormone levels in male rats

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Objectives To determine the effects of long-term cigarette smoking on the levels of plasma testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in male adult rats and to examine morphological and histological changes in the testes.

Materials and methods Cigarette smoke was generated by a smoking-machine and 12 rats were exposed to cigarette smoke diluted with 90% air for 60 days (2 h/day). Twelve rats were exposed to room air only under similar conditions as controls. The concentrations of plasma testosterone, LH and FSH were measured before and after exposure using a radioimmunoassay and the testes were examined histologically.

Results In rats exposed to smoke, the mean plasma testosterone level decreased significantly but there were no significant changes in testosterone in the control rats. The mean plasma LH and FSH levels of the two groups did not change significantly after exposure. In rats exposed to smoke, histological examination of the testes showed fewer Leydig cells and degeneration of the remaining cells.

Conclusion These results indicate that the decrease in plasma testosterone levels induced by exposure to smoke was not associated with changes in plasma gonadotrophin levels. The decrease in testosterone levels may be related to the toxic effects of smoke on Leydig cells.

Keywords Smoking, testosterone, follicle-stimulating hormone, luteinizing hormone, Leydig cell, thiocyanate, male rats

Introduction

Epidemiological studies of cigarette smokers have suggested an increased prevalence of impotence and impaired fertility. The mechanism of sexual dysfunction in men is commonly associated with low testosterone levels and some authors report that serum testosterone levels were lower in men who smoked than in non-smokers [1] but other studies have not confirmed this [2]. Therefore, the pathophysiological basis for the reduced sexual performance of smokers remains unknown. In the present study, we investigated the effects of cigarette smoking on the levels of plasma testosterone, LH and FSH and on the histology of Leydig cells in the testes of rats.

Materials and methods

Twenty-four male Wistar rats (10–12 weeks old, body weight 200–250 g) were allocated randomly into two groups; 12 rats were exposed to the smoke from 10–12 cigarettes for 2 h each day for 60 days and 12 rats were exposed to air under similar conditions, as a control. The rats were housed in stainless-steel wire cages at a constant temperature, in a 12 h light/dark cycle, with regular access to food and water.

Smoke exposure and analysis

The rats in the treated group were exposed to cigarette smoke generated using a modified Walton inhalation machine [3], comprised of three compartments; smoke was generated by lighting a cigarette in the generation chamber and one volume of smoke was mixed with nine volumes of room air in the dilution chamber. Rats in the exposure chamber inhaled this 10% mixture, provided continuously at a flow rate of 5.2 L/min. The Maltepe brand of cigarette (containing about 15 mg of tar and 0.5 mg of nicotine per cigarette) manufactured from a typical Turkish blend of tobacco was used in the experiments. Control rats were restrained in identical chambers but only exposed to room air.

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Table 1 The mean concentration of toxic compounds in the smoke-air mixture drawn from the exposure chamber

	Mean concentration (μg/m³)
Total particulate matter	1210
SO_2	387
SO_4	55
NO_2	246
NO_3	36
NH_3	100
NH_4^+	75

Samples of the smoke-air mixture were drawn from the exposure chamber and analysed spectrophotometrically; the samples contained high concentrations of toxic compounds (Table 1).

Serum analysis

Before and after exposure, blood samples were taken from all rats by cardiac puncture under ether anaesthesia and anticoagulated with heparin. Plasma was obtained by centrifuging the blood samples at 4000 ***g*** for 15 min. Plasma thiocyanate concentrations were determined using a Hitachi Model 100–20 spectrophotometer [4] and the levels of plasma testosterone, LH and FSH were measured in both groups of rats initially and again after 60 days using radioimmunoassay kits (Diagnostic Products Corp, USA) and a gamma counter.

All the animals were killed under ether anaesthesia after 60 days of exposure. The testes were removed and prepared for routine histological examination. The sections were stained with haematoxylin and eosin (H & E) and examined by one author (M.C.G.) using light microscopy.

The Wilcoxon matched-pairs signed-rank test and the Mann-Whitney *U*-test were used for statistical analysis. Probability values <0.05 were considered to indicate statistical significance.

Results

Before exposure, the mean plasma testosterone, LH and FSH levels of the control rats were not significantly different from those in the group exposed to smoke (Table 2). After exposure, the rats exposed to smoke had significantly lower mean plasma testosterone levels than the controls but there were no significant changes in the plasma testosterone level of the control rats. The mean plasma LH and FSH levels of the two groups did not change significantly after exposure (Table 2). After exposure for 60 days, the mean levels of plasma testosterone and FSH in the rats exposed to smoke were significantly lower than the corresponding values in the control rats but the mean levels of LH were not (Table 2).

Before and after exposure to smoke, the mean (SD) plasma thiocyanate concentrations were 3.7 (0.5) μmol/L and 119.0 (11.4) μmol/L, respectively ($P<0.05$), whereas the mean (SD) plasma thiocyanate levels in control rats were 3.0 (0.4) μmol/L and 3.9 (0.4) μmol/L before and after 'sham' exposure ($P>0.05$).

Histological examination of the testes showed that exposure to smoke caused degeneration of the Leydig cells and a decrease in this cell population; no such changes were observed in the control rats (Figs 1 and 2).

Discussion

Although the mean levels of plasma FSH and LH were lower than the initial values, they were not significantly so, possibly because too few animals were used in the experiments. When compared to the control rats, both testosterone and FSH levels were significantly lower in those exposed to smoke. In addition, histological examination showed that the Leydig cells had degenerated and there were fewer of them in some areas of the testes; the decrease in testosterone level was probably caused by this decrease and degeneration of the Leydig cell population.

Table 2 The plasma concentrations of testosterone, LH and FSH before and after exposure to smoke or air in exposed and control rats

	Before exposure (mean [SD])	*After exposure (mean [SD])*	*P, before versus after*	*P, with treatment*
Testosterone (pg/ml)				
Control	18.1 (1.5)	15.1 (1.9)	NS	NS*
Exposed to smoke	19.2 (1.8)	11.0 (1.7)	<0.05	<0.05**
LH (mU/L)				
Control	3.4 (1.1)	3.5 (1.6)	NS	NS
Exposed to smoke	5.4 (2.4)	1.9 (0.3)	NS	NS
FSH (mU/L)				
Control	35.5 (8.2)	36.7 (3.3)	NS	NS
Exposed to smoke	35.6 (5.4)	27.1 (1.4)	NS	<0.05

NS; Not significant. *Comparison of pre-exposure values. **Comparison of post-exposure values.

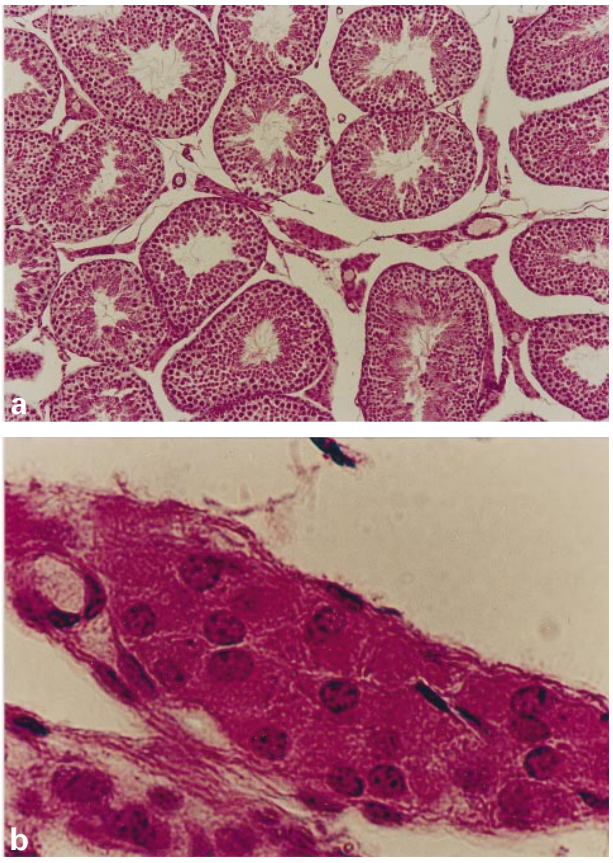

Fig. 1. Light micrographs of testicular tissue from control rats after 60 days. A normal Leydig cell population is seen in the seminiferous tubules. Haematoxylin and eosin. **a**, ×100. **b**, ×1000.

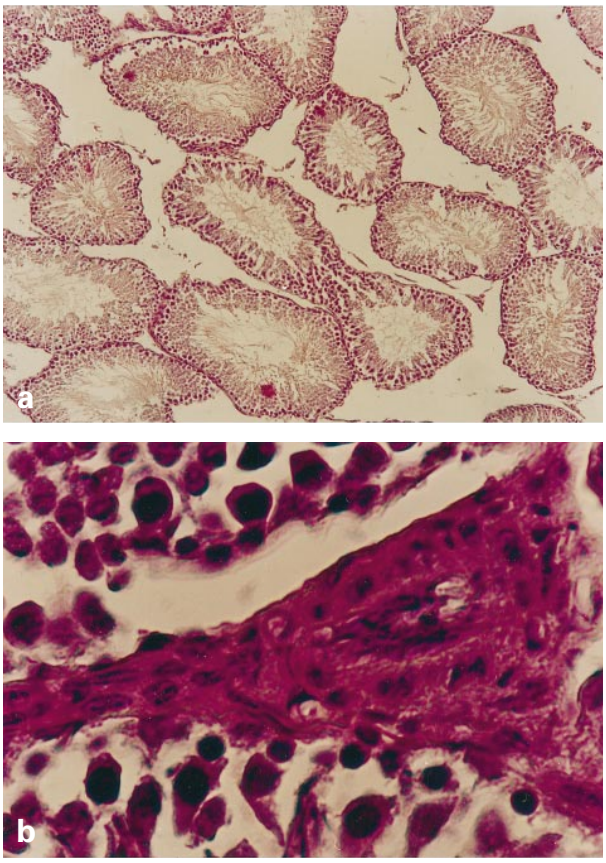

Fig. 2. Changes in the testes of rats exposed to smoke for 60 days showing the degeneration of the Leydig cells and the decrease in this cell population. Haematoxylin and eosin; **a**, ×100. **b**, ×1000.

The effect of cigarette smoking on the testes remains controversial; previous studies report conflicting results of the effects of smoking on the levels of testosterone and LH. Amatruda *et al.* [5] showed that serum testosterone levels in smokers were significantly higher than in non-smokers and similar results were obtained by Anderson *et al.* [1] and Deslypere and Wermeulen [6]. The results in these studies were attributed to the lower body weight of smokers, the amount of nicotine consumed and ethnic differences. On the other hand, the study by Shaarawy and Mahmoud [7] showed that the testosterone levels of smokers were significantly lower than those of non-smokers, with no difference in LH levels.

Many factors may affect the level of plasma testosterone, including age, dietary habits and body weight, as well as smoking [8], and animal studies have been carried out to obtain more objective results unaffected by these factors. Experimental studies on rats exposed to nicotine showed a nicotine-associated inhibition of testosterone production. It has been suggested that the effect of nicotine on the rat testis is dose-related and takes place through the acetylcholine receptors in the cell membrane and/or by direct effects of nicotine on the enzymes of Leydig-cell steroidogenesis [9,10].

In the present study, the lower levels of plasma testosterone were attributed to the degeneration of Leydig cells and a reduction in this cell population caused by exposure to smoke. The rats in the experimental group were affected significantly by cigarette smoke, reflected by the very high levels of plasma thiocyanate, which is often used in epidemiological studies because it can be measured easily using spectrophotometry and is a good indicator of the degree of smoke exposure [11,12].

Mittler *et al.* found that long-term, intense exposure to smoke in beagle dogs decreased the levels of plasma testosterone and increased those of LH. The increase of LH in these dogs probably represented a compensatory increase induced by the decreased levels of testosterone [13]. However, this particular response did not occur in the present study.

In a recent study [14], peripheral serum levels of LH, FSH and testosterone were reportedly similar in smokers and non-smokers. However, the concentration of serum

testosterone in samples aspirated from the left testicular vein was significantly lower in smokers than that in non-smokers. Thus, smoking may have a deleterious effect on the secretory function of Leydig cells which cannot necessarily be detected by determining peripheral hormone levels.

Hence, exposure to cigarette smoke seems to decrease the production of testosterone, although the mechanism(s) is still unclear. A direct effect on the Leydig cells may partly explain this toxic effect and its results in the peripheral blood. Further research, including electron microscopy, may provide a greater understanding of this effect.

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