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ORIGINAL ARTICLE

Interaction of exposure concentration and duration in determining the apoptosis of testis in rats after cigarette smoke inhalation

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Abstract The effects of differences in smoke concentration and exposure duration in Sprague Dawley rats to determine variation in type and severity of the testis apoptosis were evaluated. The daily dosages were 10, 20 and 30 non-filter cigarettes for a period of 2, 4, 6, 8 and 12 weeks. Mainstream smoke exposure suppressed body weight gain in all regimens. A dose-related increase in plasma nicotine concentration was observed in smoke-exposed groups for 4, 6, 8 and 12 week regimens. Histopathological examination of the exposed groups showed disturbances in the stages of spermatogenesis, tubules atrophying and these appeared to be dose-related. Cytoplasmic caspase-3 immunostaining was detected both in Sertoli cells and germ cells in smoke-exposure groups. An increase in TUNEL-positive cells of testicular cells was observed after 6 weeks of cigarette exposure. The results indicate that cigarette exposure concentration and duration have interaction effect to induce apoptosis in the rat testes.

1. Introduction

At present, more than 1.1 billion people worldwide are exposed to cigarette smoke (CS). Every year an estimate of 5 million people die from tobacco-related diseases (Ezzati and Lopez, 2003). CS is also well known to be reproductively toxic to males. CS and its harmful components have been documented to be associated with sperm damage such as DNA strand break (Fraga et al., 1996; La Maestra et al., 2015; Marchetti et al., 2011), DNA adducts (Potts et al., 1999), chromosomal abnormality (Yauk et al., 2007) and plasma membrane integrity (Ramlau-Hansen et al., 2007). Billig et al. (1996) think that apoptosis is supposed to be an important physiologic mechanism that limits the number of germ cells in the seminiferous epithelium of testes.

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Apoptosis is an active process by which cells are programed to die under a wide range of physiologic and developmental stimuli. Although apoptosis is an important physiologic mechanism that helps to maintain a balance between cell proliferation and cell death, a range of pathologic processes may stimulate it. An increase in apoptosis has been shown to occur in testis following experimental cryptorchidism (Shikone et al., 1994), hypophysectomy (Tapanainen et al., 1993) and exposure to toxic agents such as cocaine (Li et al., 1999) and chemotherapeutic agent such as mitomycin-C (Nakagawa et al., 1997).

In the area of inhalation toxicology, Haber's rule (Gaylor, 2000) is commonly used to estimate the relationship between the exposure dosimetry and the biological effect based on the theory that an equivalent integrated dose of concentration (C) × time (t) induces a comparable toxic effect (k). However, few studies explored the interactions of exposure concentration and duration on the apoptosis of testis. Therefore, in this study, some crucial parameters were studied to determine the influence of these parameters on the apoptosis of testis when a constant $c \times t$ dose of cigarette smoke was exposed, and to determine the relationship between cigarette smoke exposure and the degree of apoptosis in testicular cells.

2. Materials and methods

2.1. Animals

Eighty adult male Sprague Dawley rats (male, 5 weeks old) were held for 1 week in quarantine status prior to initial smoke exposure. Animals were housed 5 per cage in polypropylene boxes during the course of the experiment. The animals were maintained in a temperature (21 °C) and humidity (50%) controlled room in a 12/12 h light/dark cycle and provided with unrestricted access to water and food. This protocol was performed in a contract laboratory accredited by the American Association for Accreditation of Laboratory animals (AAALAC) and approved by the Animal Ethics Committee of First Affiliated Hospital of Xinjiang Medical University (IACUC-20131105011).

2.2. Experimental procedures

Eighty rats were divided into sixteen groups and each group has five rats. The No. 1 and No. 15 groups of rats were respectively exposed to the smoke of 10, 20 and 30 nonfilter cigarettes for a period of 2, 4, 6, 8 and 12 months. The rats in No.16 group were exposed only to filtered and humidified air, which are called sham rats.

2.3. Tobacco smoke exposures

Rats were placed in a smoking machine (model TE-10; Teague Enterprises, Davis, CA, USA) which smoked the cigarettes under the Federal Trade Commission conditions (35 mL/puff, 8 puff/min, 2 s duration). The smoke was collected through a chimney and then delivered to the whole body chambers. Mainstream cigarette smoke was determined to contribute 11% of total suspended particulate matter (TSP), which was also directed into the sidestream cigarette smoke that contributed to 89% of TSP (Yu et al., 2002). Mainstream and sidestream cigarette smoke were both introduced to a conditioning chamber and left for 2–4 min to dilute and age the smoke. The smoke content in the inhaled air was determined by continuously monitoring the concentration of TSP and carbon monoxide in the chamber for 5 h/day, 5 days/week.

After tobacco smoke exposure, each rat was given 0.4 mL/100 g of sodium pentobarbital. Blood of rats was collected into ethylenediaminetetraacetic (EDTA) tubes and then centrifuged for 30 min. Aliquots of the plasma specimens were stored at − 80 °C and were ready to use in the following nicotine and cotinine concentration determination. All rats were observed daily and the general states were recorded. Body weights (BW) of rats were measured weekly since the initial day of smoke exposure.

2.4. Concentrations of nicotine and cotinine in plasma

The concentrations of nicotine and cotinine in plasma were determined using the method reported by Massadeh et al. (2009). A 2.5 mL aliquot of dichloromethane–hexane (1:1 v/v) was used for one-step extraction. Plasma samples were alkalinized with 100 μL of 2.5 M NaOH and then a 0.5 mL aliquot of plasma was added into a screw-capped tube with 100 μL of internal standard (2 ppm diphenylamine in 50% methanol). The organic layer was transferred to a new tube which contained 10 μL of glacial acetic acid, and then vortex mixed at 3500 rpm for 3 min. The organic phase was evaporated under a stream of nitrogen at 35 °C until dryness, and then was reconstituted to 100 μL with hexane. A 2 μL aliquot was injected manually into the GC–MS. The operational parameters were: injector temperature of 300 °C and transfer line temperature of 300 °C. Oven temperature was programed from 120 to 220 °C (20 °C/min), and then held for 2 min. The selected ions were: m/z 162 for nicotine and 176 for cotinine.

2.5. Immunohistochemistry analyses

Immunohistochemical analyses of anti-caspase-3 primary antibodies were performed to detect apoptosis. The formalin-fixed

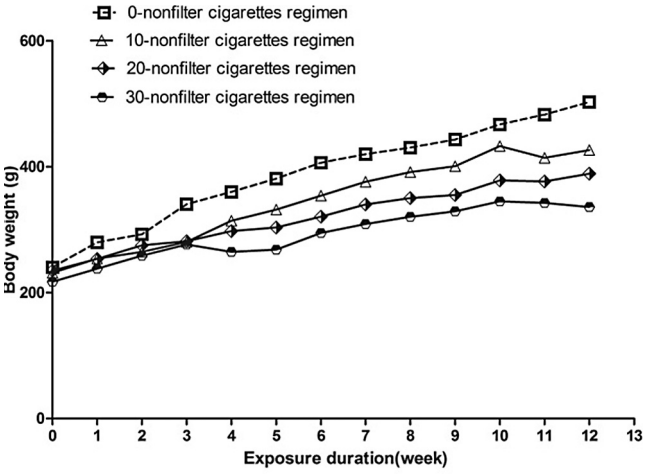

Figure 1 Body weight. Rats were sham exposed or were exposed to different doses of cigarettes over 12 weeks. Error bars have been omitted for clarity (mean, n = 5 per group).