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The morphology and mineralization of dental hard tissue in the offspring of passive smoking rats

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ABSTRACT

Objective: To study the effects of maternal passive smoking on the morphology and mineralization of dental hard tissue in offspring rats.
Design: We have established a maternal passive smoking model. Offspring rats were sacrificed on the 20th day of gestation (E20) or the 3rd (D3) or 10th day (D10) after birth. We observed hard tissue morphology using Haematoxylin–Eosin (H&E) staining sections, used micro computer tomography (Micro-CT) to measure hard tissue thickness and volume on the mandibular first molars of the offspring rats, and used Micro-CT and energy dispersive X-ray spectroscopy with scanning electron microscopy (SEM/EDS) to determine the hard tissue mineral density and the ratio of calcium atom number/calcium atom + phosphorus atom number ($Ca^{2+}/P^{3-} + Ca^{2+}$).
Results: Overall, the development of dental hard tissue was delayed in the offspring of passive smoking rats. The thickness and volume of hard tissue were lower in the offspring of the maternal passive smoking group than in the offspring of the control group. Mineral density of the hard tissue and the ratio of ($Ca^{2+}/P^{3-} + Ca^{2+}$) were also reduced in the offspring of the maternal passive smoking group.
Conclusion: Maternal passive smoking inhibits the morphological development and mineralization level of hard tissue on the mandibular first molars of offspring rats.

1. Introduction

Tobacco contains more than 4000 ingredients, and the fumes produced by its combustion contain a large number of toxic substances, including nicotine, tar, carbon monoxide, cyanide and radioactive substances. Both active and passive smokers inhale tobacco smoke; when inhaled by a pregnant woman, tobacco toxins can cross the placental barrier and poison many organs and tissues of the foetus. The overall teratogenic effect of tobacco smoke is uncertain, but a positive association with maternal smoking has been reported for congenital heart defects,[1] limb reduction defects,[2] kidney malformations,[3] and oral clefts.[4] Evidence also shows that passive smoking causes the following diseases and conditions: PPHN (persistent pulmonary hypertension of the newborn),[5] lower birth–weight babies,[6] bronchitis,[7] middle ear disease,[8] and even SIDS (Sudden Infant Death Syndrome).[9]

Since the 1980s, some epidemiologists have expressed concern about the relationship between maternal passive smoking and offspring tooth development. Heikkinen et al. collected the data on primary tooth crown dimensions of 5–12 years-old children and found 2–3% reductions mesio-distally and labio-lingually in the teeth of offspring of smoking mothers. In addition reduced crown dimensions were found

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also in permanent first molars and incisors, which were ''thin'' in labio-lingual crown dimension.[10–12] Other epidemiological surveys have reached similar conclusions.[13,14]

A number of laboratory studies have also examined this issue. Khan et al. cultured mandibular first molars extirpated from 18-day-old mouse foetuses. Two-day cultures were treated with 78, 117, 156, or 312 μg/ml nicotine sulphate. Tooth germs treated with 78 μg/ml nicotine were only slightly affected, but higher doses of the drug produced extensive cell damage.[15] Chowdhury et al. measured the length, width and occlusal areas of the first maxillary molar crowns of laboratory rats exposed to nicotine. It was found that, when compared to control rats, dental asymmetries were significantly increased whilst occlusal areas were significantly decreased in nicotine-exposed rats.[16]

Recently, many scholars have examined the relationship between maternal smoking and the rate of offspring dental caries. Aligne et al. collected cross-sectional data from the Third National Health and Nutrition Examination Survey of 3531 children between the ages of 4 and 11 years; the children underwent both dental examinations and the measurement of serum cotinine levels. The study found an association between environmental tobacco smoke and the risk of dental caries amongst children.[17] Tanaka et al. studied 3-year-old children whose mothers were exposed to environmental tobacco smoke (ETS) during pregnancy or 3-year-old the children who postnatally exposed to ETS. They concluded that both in utero exposure to maternal smoking and postnatal exposure to ETS may be associated with an increased prevalence of dental caries in young children. They concluded that both in utero exposure to maternal smoking and postnatal exposure to ETS may be associated with an increased prevalence of dental caries in young children.[18] These results indicate that passive smoking may affect the quality of offspring dental hard tissue; it is necessary to further study whether dental hard tissue mineralization is also affected by maternal passive smoking.

In summary, most previous studies have either examined epidemiological data or have been laboratory studies focused on the effects of nicotine injection rather than the effects of inhaled cigarette smoke, which contains a variety of compounds in addition to nicotine. A simulative passive smoking animal model measuring the effects of smoke on offspring tooth development has not been established. Previous experiments were also more concerned about changes in tooth germ cells than in hard tissue, and they lack quantitative hard tissue measurements. More importantly, no study has investigated how the degree of mineralization of hard tissue is affected by maternal passive smoking. Tooth development is a continuous process of mineralization, and few studies have paid attention to the total stage of hard tissue formation. Our study hopes to renew these research endeavours and to fill those gaps.

The aim of the current study was to establish a passive model simulating maternal smoking and to examine differences in dental hard tissue morphology and mineralization between the offspring of passive smoking and non-passive smoking rats at several time points.

2. Materials and methods

2.1. Animal maintenance and genotyping

80 healthy adult SD rats (60 female and 20 male from the Experimental Animal Centre of Sichuan University, Chengdu) were maintained in a virus and parasite-free barrier facility and fed a standard diet. Females had no reproductive history. The rats were mated in accordance with the sex ratio of 3:1 and vaginal suppository was checked the morning after mating. Successfully mated pregnant rats were randomly divided into two groups: 30 in the control group and 30 in the passive smoking group. Rats in the passive smoking group were placed in a passive smoking device (an airtight glass box measuring 80 mm × 60 mm × 60 mm). Tobacco smoke was given 2 h twice per day until the day of childbirth (SPHERE® tobacco from the same batch produced by British-American Tobacco). 10 rats from each group were sacrificed on the 20th day of gestation (E20), and 5 embryos from each rat were removed from the uterus, and sacrificed. 10 rats from each group were randomly selected and 5 offspring rats born by each mother were sacrificed on the 3rd day after birth (D3). 10 rats in each group were left and 5 offspring rats from the same mother were sacrificed on the 10th day after birth (D10).

2.2. H&E staining

Mandibles on one side in the offspring rats were dissected and fixed in 4% paraformaldehyde for 24 h, decalcified in EDTA for 4 days, embedded in paraffin, sectioned parallel to mandibular axes at 5 μm, and stained with conventional Haematoxylin–Eosin. The histological features of the sections were examined by light microscope.

2.3. Micro-CT

The tooth germs were removed from the mandible on the other side with the stereomicroscope and fixed in 4% paraformaldehyde for 24 h (Fig. 1). A Micro-CT system(μCT80 Micro-CT produced by SCANCO) with analysis software was calibrated with a control disc made of hydroxyapatite. Parameters selected for this study included a source voltage of 55 kV and current of 170 μA. Each scan yielded an image data set of 1024 × 1024 2D axial slices acquired in 10 μm sections. We chose the most shape-standard 10 slices to measure hard tissue thickness: on each slice, a hard tissue image from 3 cusp tips to the top were divided into three equal portions by the 6 trisection points (marked a, b, c, d, e and f) (Fig. 2); the average length at the six points represented the hard tissue thickness. Then we adjusted the threshold to 132, using a three-dimensional reconstruction system to obtain three-dimensional images of dental hard tissue; the hard tissue volume and mineral density were simultaneously obtained from the analysis software.

2.4. SEM/EDS

A SEM/EDS system (HITACHIS3400) was used in the study. The samples used with Micro-CT were cycled through distilled water, 70%, 90%, 100% alcohol, and xylenes; they were then