Journal of Cell and Molecular Research (2011) 3 (1), 38-42

Maternal nicotine exposure-induced collagen pulmonary changes in Balb/C mice offspring's

Mohammad Reza Nikravesh[1], Mehdi Jalali[1], Abbas Ali Moeen[2], Shabnam Mohammadi[1*] and Mohammad Hasan Karimfar[2]

[1]*Department of Anatomy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Department of Anatomy, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran*

Received 16 February 2011 Accepted 5 June 2011

Abstract

Nicotine is an alkaloid by high level of addictive property that can quickly assimilate from smoker's lung. It passes from the placenta and gathers in the developing fetus. Our previous study showed that collagen type IV plays a critical role in basement membrane of different embryonic organs. In this study the effect of maternal nicotine was evaluated by collagen IV changes in lung of mice offspring during pre and postnatal period. Pregnant Balb/C mice were divided into 2 experimental and 2 control groups. Experimental group 1 received *3 mg/kg* nicotine intrapritoneally from day 5 of gestation to last day of pregnancy. Experimental group 2 received the same amount of nicotine during the same gestational days as well as 2 first week after birth. The control groups received the same volume of normal saline during the same periods. At the end of exposure times, all newborns were anesthetized and their lungs were removed and immunohistochemical study for tracing collagen was carried out. Our results showed that collagen reaction in the bronchial basement membrane (BBM) and extra cellular matrix (ECM) of the lung parenchyma experienced a remarkable increase when compared to the control ones. Cell necrosis definition in lung parenchyma of the experimental group 2 was the other finding that our investigation revealed. These data indicate that maternal nicotine exposure may induce a noticeable collagen increase with a reasonable amount in BBM and ECM of respiratory system of next generation.

***Keywords*:** respiratory system, nicotine, collagen IV, mouse

Introduction

Nicotine is an alkaloid obtainable from tobacco plant. It is one of the most important components of cigarette by high level of addictive property (Martin, 1970). Nicotine is a lucid liquid with an unpleasant odor that, when exposed to air, changes to brown (Catassi et al., 2008). Some of studies indicate that nicotine passes quickly from placenta and gets accumulated in the fetus and causes adverse effects on fetus development (Sung-HwaSohn et al., 2008; Harmanjatinder et al., 2002; Taylor and Wadsworth, 1987). On the other hand, other studies show that nicotine causes growth retardation and decreases birth weight (Wen et al., 1990; Cliver et al, 1995; Vogt, 2004).

By increasing cigarette smoking in society, especially in young woman, it is necessary to investigate the effects of maternal nicotine exposure during lung development of the offspring. Our previous results indicated that collagen IV expression plays an important role in formation of retina (Nikravesh et al., 2009). Another investigation also revealed that anterior epithelium development and matrix of the lens, especially its marginal zone, are dependent on this molecule (Nikravesh et al., 2009; Jalali et al, 2009). Also, our previous studies (Karimfar et al., 2009; Nikravesh et al., 2009) implicated that the appearance of the collagen type IV during tubule and glomeruli morphogenesis represents that this molecule contribute to nephrogenesis during urinary tract formation (Jalali et al., 2009; Moein et al., 2008). Also, its role in brain choroid plexus (BCP) development indicated that formation of vascular plexus is dependent on collagen type IV, main structural component of the BM (Nikravesh et al., 2009). It seems that factors affecting the collagen regulation during lung development may put the normal health of the respiratory system at risk (West, 2009; Kang et al., 2009; Hinenoya et al., 2008; Lan et al., 2008). Hence, the aim of this study was to investigate the effects of maternal nicotine exposure on lung connective tissue

∗Corresponding author E-mail:
shabnammhmmd@yahoo.com

development especially collagen type IV, of the mouse offspring.

Materials and Methods

Nicotine administration and tissue preparation

Twenty four female Balbc/c mice were divided randomly into 2 experimental and 2 control groups and appearance of the vaginal plug was designated as day zero of pregnancy. The environmental conditions were 22±1°C and 12 h light-dark cycle with free access to water and food. The experimental group 1 (n=6) injected daily intraperitoneal dose of 3 mg/kg of nicotine from day 5 of gestation to the last day of pregnancy (Hisa et al., 2003). Experimental group (n=6) were received the same amount of the nicotine during gestation and two weeks after birth (lactation). The control groups (n=12) were received nicotine solvent (Normal saline). Finally, the animals were rapidly sacrificed by cervical dislocation and the lung of the mice were removed and fixed for 24 hours at room temperature in formaldehyde 10% and immunohistochemistry study for tracing collagen type IV were carried out.

Immunohistochemistry study

The Avidin-Biotin peroxidase procedure was used for immunohistochemistry study. Sections washed twice for 5 min in 0.05 Tris buffer containing 1.5% NaCl, pH 7.4. For blocking the nonspecific antibody reactions, the sections were preincubated in 0.3% Triton X-100 in TB-NaCl followed by 5% goat serum for 1-2 h. Then they were reacted for 12-24 h at 4°C with primary antibody anti-collagen IV monoclonal antibody (Sigma-Aldrich, USA), diluted 1:50 in TB-NaCl with 0.3% Triton and 2% serum. Tissues were washed with TB-NaCl for three times, each time for 10 min, and incubated for 2 h in biotinylated goat anti-rabbit IgG (1:400 in TB-NaCl). After three further rinses, for 1 h each time, endogenous peroxidase activity was blocked by their incubation in 0.03% H_2O_2 in methanol for 30 min. Tissues were incubated for 2 h in 1:100 avidin-biotinylated horseradish peroxidase complex. Then they were washed three times, each time for 30 min in TB-NaCl, and finally reacted with 0.03% solution of 3, 3-diaminobenzidine tetrahydrochloride for 10-15 min. Tissues containing 0.03% H_2O_2 were washed and lightly counterstained with hematoxylin. Subsequently, they were washed and mounted in PBS glycerol. Collagen reaction in BM of alveolus and lung parenchyma was graded by a sampling computerized method.

Results

Tracing of collagen in different parts of the lung indicated a weak reaction in the alveolar basement membrane in our experimental groups. However this reaction was not significant while compared to the control groups (figures 1a, 1b). These reactions increased to dark brown in the alveolar basement membrane in the experimental groups (table 1) and although collagen synthesis of the BM did not show significant change in experimental groups, the collagen reaction increased remarkably in comparison to the control groups (figure 1c, 1d). Collagen appeared as light brown color in the extracellular matrix in the control groups (figure 2a, 2b). The intensity of reaction in extracellular matrix of lung parenchyma in the experimental groups increased significantly compared to the control groups (figure 2c, 2d). Besides, remarkable signs of picnotic nucleuses and cell death in lung parenchyma in experimental group 2 were observed, but these changes were not noticeable in the experimental group 1 and that of control.

Discussion

Previous studies have shown that ECM and BM play important roles in lung developmental process. Basement membrane is a specialized region of the extracellular matrix consisting of multiple matrix molecules and plays a major regulatory role in developmental phenomena of proliferation, morphogenesis and migration. Among the components of the BM, collagen type IV is the most important parts of this region. Results of this study indicated that collagen increased significantly in the basement membrane (BM) of the lung alveolar in experimental groups as well as lung parenchyma.

These data indicate that although collagen synthesis of the BM did not show significant change in the experimental groups, the reaction remarkably increased. Besides, collagen fibers in the experimental group 2 significantly increased when compared to the experimental group 1 and even some signs of necrosis and cell death in lung parenchyma of experimental group 2 was detectable So, it seems that the lungs of these newborns, exposed to nicotine via the placenta and mother's milk, are more susceptible to damages such as abnormal collagen synthesis and cell necrosis. Our previous studies showed that collagen type IV is a major protein in many developmental processes. The results of this study showed that maternal nicotine exposure leads to collagen changes and basement membrane in the lungs of

their offspring. In mice pups, exposed to nicotine during pregnancy, as well as lactation the collagen fibers showed an increase in basement membrane of respiratory tract and extra cellular matrix.

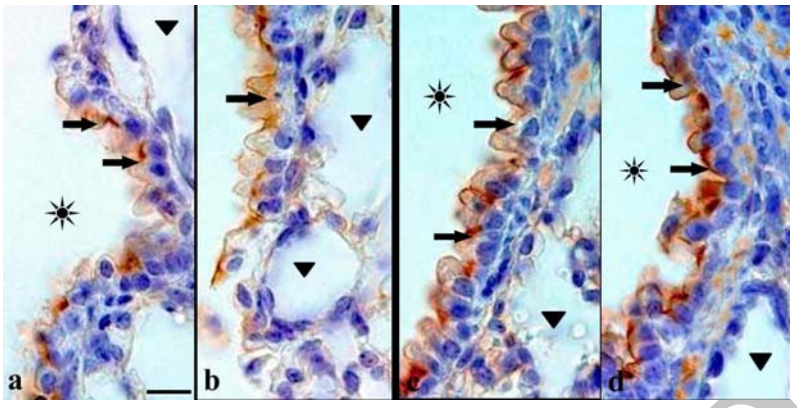

Figure 1. Sections through the respiratory bronchioles of the 14-day old mice that incubated with antibody against collagen type IV in control group 1 (a), control group 2 (b), experimental group 1 (c), and experimental group 2 (d). The respiratory tract lined with columnar epithelium and arrows indicate basement membrane. In these sections terminal bronchiole (asterisks) and lung alveolar (arrowheads) are visible (scale bar=100 µm, Hematoxylin counterstained).

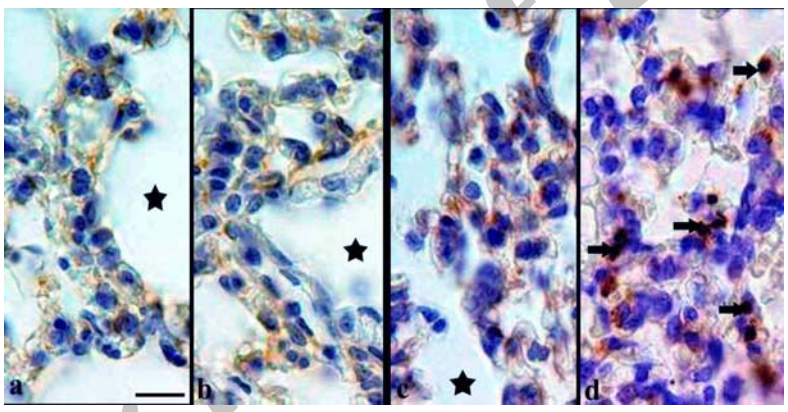

Figure 2. Sections through the lung parenchyma of the 14-day rats. These sections were prepared from control group 1 (a), control group 2 (b), experimental group 1 (c), and experimental group 2 (d) which are incubated with antibody against collagen type IV. The epithelial cells with irregular arrangement and ECM are visible from light to dark brown (arrows). In these sections the terminal bronchiole (asterisks) is visible. Besides to the alveolar cells, cell necroses are obvious (arrows) in the experimental group 2. The micrographs include a scale bar (=100 µm) with Haematoxylin counterstaining.

Table 1. Comparison between lung parenchyma parameters in the experimental and control groups

	Control group 1	**Experimental group 1**	**Control group 2**	**Experimental group 2**
Collagen reaction	**(++)**	**(++++)**	**(++)**	**(++++)**

The intensity of the reaction is assigned by + with the following grades: negative (-), weak (+), moderate (++), strong (+++) and highly strong (++++).

In the exposed animals complications such as cell necrosis in lung parenchyma were also obvious. A cause of this may be the higher level of lipids and acidic property in mother's milk than its serum. As nicotine level in animals, exposed to nicotine via mother's milk, was already shown to be two or three times higher than that of plasma (Gert, 1988).

Studies show a suppression of glycolysis could occurre in lung of animals that are exposed to nicotine. Because type I pneumocytes are dependent on glycolysis and type-II pneumocytes proliferate into type-I ones (Maritz, 1995; Martiz, 1985). It is possible that any change in this phenomenon may lead to a disturbance in programed cell death (Johannes et al., 1998). Although we should not ignore the effect of nicotine on glycolysis, probably nicotine can also induce lipid peroxidation that decreases the antioxidant capacity of the lung. Oxidant/antioxidant imbalance could, in turn, change the genetic program of the genes involved in glycolysis or synthesis of proteins such as collagen type IV.

Acknowledgment

This study was a collaborative research project of Mashhad University of Medical Sciences and Zabol University of Medical Sciences. It was funded by Zabol University research deputy. We thank both Mashhad and Zabol Universities of Medical Sciences for their cooperation. Also, we are grateful to Ms Motajadded from histology laboratory of the medical school for her technical assistance.

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