Prenatal Nicotine Increases Matrix Metalloproteinase 2 (MMP-2) Expression in Fetal Guinea Pig Hearts

Reproductive Sciences
18(11) 1103-1110
© The Author(s) 2011
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719111404605
<http://rs.sagepub.com>

Loren P. Thompson, PhD¹, Hongshan Liu, MD¹,
LaShauna Evans, BS², and Jessica A. Mong, PhD³

Abstract

This study tested the hypothesis that maternal nicotine ingestion increases matrix metalloproteinase (MMP) expression in fetal hearts, which is mediated by the generation of reactive oxygen species. Timed pregnant guinea pigs were administered either water alone, nicotine (200 µg/mL), *N*-acetylcysteine (NAC), or nicotine plus NAC in their drinking water for 10 days at 52-day gestation (term = 65 days). Near-term (62 days), anesthetized fetuses were extracted, hearts were excised, and left cardiac ventricles snap frozen for analysis of MMP-2/-9/-13 protein and activity levels. Interstitial collagens were identified by Picrosirius red stain to assess changes in the extracellular matrix. Prenatal nicotine increased active MMP-2 forms and interstitial collagen but had no effect on either pro- or active MMP-9 or MMP-13 forms. In the presence of nicotine, NAC decreased active MMP-2 protein levels and reversed the nicotine-induced increase in collagen staining. We conclude that prenatal nicotine alters MMP-2 expression in fetal hearts that may be mediated by reactive oxygen species generation.

Keywords

collagen, cardiac remodeling, reactive oxygen species, *N*-acetylcysteine

Introduction

Maternal smoking is a major risk factor to the health and well-being of both the mother and fetus. The incidence of pregnant women who smoke cigarettes is 18% (age 15-44 years old) compared to 30% of age-matched nonpregnant women, which varies with ethnicity, education, and economic status.¹ Many start as teenagers and continue to smoke when they become pregnant. In the first trimester, maternal smoking contributes to increased incidence of preterm delivery, spontaneous abortions and stillbirths, and in the second and third trimester, to intrauterine growth restriction and altered fetal organ growth and function.² Long-term effects of maternal smoking and or exposure to environmental tobacco smoke during pregnancy in the neonate is associated with several neurological deficits including behavioral problems, cognitive impairment, addiction, and depression³ as well as cardiorespiratory problems contributing to sudden infant death syndrome (SIDS).^{4,5}

Nicotine is one of the major bioactive components in tobacco smoke and is both addictive^{6,7} and may increase cancer risk through activation of nicotine acetylcholine receptors.^{7,8} In addition, nicotine exposure contributes to cardiovascular disorders such as cardiac arrhythmias, hypertension, and vasospasm, via both direct and indirect mechanisms.^{9,10} These may be mediated by nicotine's direct interaction with cardiac inward rectifier K⁺ channels,¹¹ which may alter cardiac action potential or indirectly, by stimulating catecholamine release from the adrenal medulla⁹ and sympathetic nerve endings.^{9,12,13}

Following maternal ingestion, nicotine readily crosses the placenta and concentrates in the fetal circulation,² having diverse effects in fetal organs. These include decreases in blood flow in brain, synaptic function, and neuronal cell proliferation and differentiation.¹⁴ In the developing fetal lung, nicotine inhibits maturation and disrupts alveolar number, structure, and function.¹⁵ Prenatal exposure to nicotine has also been shown to increase contractile responsiveness of the aortae to norepinephrine and KCl^{16,17} and increase the susceptibility to ischemia/reperfusion injury in male rat offspring.¹⁸ Recent study indicates that prenatal nicotine exposure represses gene expression of protein kinase C epsilon in fetal rat hearts contributing to programming of the adult offspring.¹⁹ These studies suggest that prenatal nicotine initiates specific pathological responses that both impact the fetus and have lasting consequences in the neonate.

¹ Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland, Baltimore, MD, USA

² Department of Physiology, University of Maryland, Baltimore, MD, USA

³ Department of Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, MD, USA

Corresponding Author:

Loren P. Thompson, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland, Baltimore, Bressler Research Bldg., Rm 11-029, 655 W. Baltimore St, Baltimore, MD 21201, USA
Email: lthompson1@umm.edu

In adult hearts, nicotine exposure is associated with increased proinflammatory cytokines,⁹ oxidative stress,²⁰⁻²⁴ and matrix metalloproteinases (MMPs).^{25,26} Both the inflammatory response and oxidative stress have been shown to favor the activation of MMPs as well as regulating gene transcription in heart tissue.^{26,27} Metalloproteinases are important enzymes that contribute to cardiac remodeling.^{25,26} While there are at least 28 subtypes, MMP-2 and -9, or gelatinase A and B, respectively, and MMP-13, a collagenase, are the predominant MMPs expressed in cardiac ventricles,²⁶ which regulate the structural integrity of the extracellular matrix (ECM). Altered MMP expression is associated with disruption of the extracellular network, which has been shown to contribute to structural remodeling in pathological heart tissue.²⁶

The purpose of the present study is to investigate the effects of prenatal nicotine exposure on MMP expression of fetal heart ventricles. We hypothesize that nicotine exposure upregulates MMP expression as an important signaling pathway of fetal heart injury, which may be manifested in the ECM. This could have both short- and long-term consequences in the fetal heart resulting in lasting effects in the offspring.

Materials and Methods

Animal Model

Timed pregnant Dunkin-Hartley guinea pigs (term = 65 days) were administered either water (control, N = 7), or nicotine bitartrate dehydrate (200 µg/mL, N = 4) in the drinking water for 10 days at 52-day gestation. The average nicotine intake was 20.0 ± 2.4 mg/kg per d, well within the reported range of 7 to 60 mg/kg per d, known to increase plasma nicotine levels achieved by habitual smokers.^{28,29} Food (g/d; Harlan Teklad Guinea Pig Diet 7006 Harlan Laboratories, Inc., Indianapolis, IN) and fluid intake (mL/d) were monitored every other day during the treatment periods. To test the effect of oxidative stress on fetal hearts, *N*-acetylcysteine (NAC; 10 mg/mL, N = 4) was administered in the drinking water in the presence/absence of nicotine at a dose (496 ± 37 mg/kg per d) that was effective (300-500 mg/kg) in inhibiting oxidative stress.³⁰⁻³² *N*-Acetylcysteine is an endogenous substrate in glutathione synthesis, a natural antioxidant via the glutathione pathway,³³ and considered safe during pregnancy.³⁴ At near term, pregnant animals were anesthetized with ketamine (80 mg/kg) and xylazine (1 mg/kg) and administered a subcutaneous injection of lidocaine prior to receiving an abdominal incision. Age-matched fetuses (62 days, term = 65 days) were excised via hysterotomy. Fetal body, placenta, heart, and brain weights were measured and relative organ weights normalized to their respective fetal body weights were calculated. Fetal blood was obtained via cardiac puncture from anesthetized fetuses via a 25-gauge syringe needle and a 1-mL tuberculin syringe. Fetal serum cotinine levels were measured (Cotinine Direct ELISA, Bio-Quant, San Diego, California) as an index of fetal nicotine distribution following maternal ingestion. The methods used were approved by the University of Maryland Animal Care and Use Committee and conform to the

Guide for the Care and Use of Laboratory Animals published by US NIH Publication No. 85-23, 1996.

Quantification of MMP Protein Levels by Western Analysis

Protein levels of MMP-2, -9, and -13 were quantified using Western blot analysis similar to that previously described.³⁵ Fetal hearts of control, nicotine-, and nicotine + NAC-treated animals were weighed, left ventricles excised, and immediately frozen in liquid N₂ and stored at -80°C until ready for study. Frozen left ventricle sections of fetal hearts were homogenized in ice-cold lysis buffer (0.5 mol/L Tris-Cl, pH 7.4, 1.5 mol/L NaCl, 10 mmol/L ethylenediaminetetraacetic acid [EDTA], 2.5% deoxycholic acid, 10% NP-40, protease inhibitor, and phosphatase inhibitor), placed on ice for 1 hour, and spun at 13 000g at 4°C for 10 minutes. Protein concentration of the supernatant was analyzed by the Bradford Protein Assay (Bio-Rad Laboratories, Hercules, California). Equal amounts (40 µg) of protein of each group were loaded onto 4% to 15% Tris-HCl polyacrylamide gels and separated by gel electrophoresis. Proteins were transferred to Immun-Blot poly(vinylidene fluoride) (PVDF) membranes (Bio-Rad Laboratories), blocked for 1 hour at room temperature and probed overnight at 4°C. Membranes were incubated with a polyclonal antibody specific for MMP-2, -9, and -13 (1:1000, Chemicon International Inc, Temecula, California) and followed by the second antibody (1:10 000, horseradish peroxidase-conjugated goat anti-rabbit for MMP-2/-9 and bovine antigoat for MMP-13; Santa Cruz Biotechnology, Inc, Santa Cruz, California) after extensive washing. Protein bands were detected by ECL Western Blotting Analysis System (Amersham, Piscataway, New Jersey). Density values of each band were determined by densitometry (UN-SCAN-IT gel Automated Digitizing System V5.1; 32-bit; Skill Scientific Corporation, Orem, Utah) and normalized to α -actin or glyceraldehyde 3-phosphate dehydrogenase ([GAPDH]; 1:1000, Abcam, Inc, Cambridge, Massachusetts) as a loading control.

Quantification of Pro and Active Forms of MMP by Gelatin Zymography

Gelatin zymography was performed to quantify the proteolytic activity of MMP-2 and -9 as reported previously.³⁵ Briefly, frozen fetal guinea pig left ventricles of control and nicotine-treated animal groups were homogenized and mixed with loading buffer and 40 µg were loaded onto 10% zymogram Ready Gel containing 10% gelatin (BioRad Laboratories). Gels were electrophoresed for 90 minutes at 125 V, then incubated in 1× renaturing buffer for 20 minutes × 3 at room temperature. Gels were subsequently incubated in 1× developing buffer overnight at 37°C. To visualize the bands, gels were stained with 2% Coomassie Blue for 1 hour at room temperature and destained for 2 to 4 hours using 40% methanol, 10% acetic acid, and 50% H₂O. Zymograms were analyzed by scanning the destained gels on a KODAK Gel Logic 440 Imaging

System (Carestream Health, Inc., New Haven, Connecticut) with a light conversion software program (KODAK White Light Padd). The light bands on a dark blue background were converted into dark bands and arbitrary density values were obtained for analysis using UN-SCAN-IT gel Automated Digitizing System V5.1 (32-bit; Skill Scientific Corporation) software program for comparison between groups. Bands identified both pro and active forms of MMP-2 based on the appropriate molecular weights. Bands corresponding to MMP-9 were absent.

Analysis of Myocardial Interstitial Collagen Content

Staining for myocardial interstitial collagen was performed on fetal heart ventricles using 0.1% (g/mL) Sirius red stain in saturated picric acid (Picrosirius).³⁶ Fetal hearts were obtained from control, nicotine, NAC alone, and nicotine + NAC animal groups and immediately immersed in 10% buffered neutral formalin and allowed to fix at least 24 hours prior to processing. After being fully fixed, tissues were embedded in paraffin and sectioned at 5 μ m. After deparaffinization and rehydration, sections were stained with Picrosirius red stain (0.1%) for 1 hour for detection of collagen fibers. Slides were washed twice with acidified water, shaken to remove excess water, and dehydrated using 3 changes of ethanol (70% to 100% ethanol). Slides were then cleared with xylene and mounted in resinous medium for viewing.

Statistics

The number of fetuses used in each group is represented by N values. Each fetus was obtained from a different sow. Responses are expressed as mean $\pm$ standard error of the mean. Analysis of variance (ANOVA) was used to compare the differences among the 4 treatment groups using a post hoc analysis (Student-Newman-Keuls Method) to determine the significance between groups with a $P < .05$. Collagen fiber staining with Picrosirius red stain was quantified from transverse sections of fetal hearts sectioned at the equator to include both right and left ventricles. Positive staining was detected from imaging 6 random areas within each section devoid of blood vessels and quantified using NIH Image J software (version 1.57; <http://rsb.info.nih.gov>). The average value of cytosolic staining per area was obtained for each treatment and compared using ANOVA followed by post hoc analysis (Student-Newman-Keuls Method) for identifying difference between groups ($P < .05$).

Results

Nicotine Administration

The average maternal nicotine intake rate was 20.0 ± 2.4 mg/kg per d and associated with fetal serum cotinine levels of 85 ± 5 ng/mL. Cotinine was undetectable in untreated fetuses. Fetal cotinine levels were within the human levels (60–200 ng/mL cotinine) measured in moderate, habitual smokers,^{28,29} indicating

sufficient nicotine exposure of the fetus. Cotinine is the metabolite of nicotine and is the preferred index of measurement for long-term administration because of its long half-life compared to nicotine.²⁸

Fetal Weight Characteristics

Prenatal nicotine increased ($P < .05$) both food (41.0 ± 2.0 mg/d vs 55.6 ± 1.9 mg/d, control vs nicotine) and fluid (95.7 ± 8.9 mL/d vs 133.4 ± 12.0 mL/d, control vs nicotine) intake compared to control animals. Despite these differences, nicotine had no significant effect (control vs nicotine) on fetal body weight (76.9 ± 2.2 g vs 78.2 ± 4.5 g), placental/body weight ratios (0.063 ± 0.002 vs 0.059 ± 0.002), heart/body weight ratios (0.0058 ± 0.0001 vs 0.0063 ± 0.0002), or brain/body weight ratios (0.033 ± 0.001 vs 0.035 ± 0.002). *N*-Acetylcysteine plus nicotine significantly ($P < .05$) reduced both food (34.2 ± 2.1 mg/d) and fluid (50.2 ± 2.1 mL/d) intake compared to nicotine alone but had no effect on fetal body weight (78.2 ± 4.5 g vs 65.8 ± 3.8 g, nicotine vs nicotine + NAC). In addition, NAC plus nicotine had no effect on relative placental weight (0.059 ± 0.002 vs 0.063 ± 0.003 , nicotine vs nicotine + NAC), heart weight (0.0063 ± 0.0002 vs 0.0057 ± 0.0002), or brain weight (0.035 ± 0.002 vs 0.038 ± 0.002) compared to nicotine alone.

Effect of Nicotine on Cardiac MMP Protein Expression

Figure 1 illustrates both the immunoblot of individual heart samples and the average protein levels for control and nicotine-treated animals. The density value of each band was normalized to its corresponding α -actin value and graphed as the average of the group in Figure 1. Prenatal nicotine significantly ($P < .05$) increased MMP-2 protein levels by 36.3% compared to its untreated controls.

Matrix metalloproteinase 2 protein is synthesized intracellularly and subsequently released as the pro form and activated (active form) in the interstitial space.²⁶ Identified by gel zymography, Figure 1 illustrates both pro (~ 72 kDa) and active (~ 62 kDa) forms of MMP-2 in fetal heart tissues. Prenatal nicotine had no effect on pro form levels but significantly ($P < .05$) increased active MMP-2 levels by 45.5%.

Figure 2 illustrates the effect of nicotine on protein expression of MMP-9 and MMP-13 of fetal heart ventricles by Western analysis. Prenatal nicotine had no significant effect on either pro or active forms of MMP-9 or MMP-13 compared to the untreated controls, although the active protein levels were significantly greater than the pro forms.

Effect of NAC on Cardiac MMP Expression

To test whether nicotine-induced changes in MMP expression are mediated by reactive oxygen species (ROS), we measured MMP-2 protein levels in fetal hearts of nicotine-treated animals with or without NAC. In Figure 3, NAC significantly ($P < .05$) reduced MMP-2 protein levels by 31.1% compared to animals

Figure 1. Western analysis (left) and gelatin zymography (right) of matrix metalloproteinase 2 (MMP-2) of control (untreated, N = 4) and nicotine-treated (N = 4) fetal heart ventricles. Matrix metalloproteinase 2 bands were identified in Western immunoblots at ~72 kDa and normalized to bands corresponding to α -actin as a loading control. Gel zymography identified both pro (72 kDa) and active (62 kDa) bands for MMP-2. Band densities were quantified by densitometry and expressed as arbitrary values. Values are mean $\pm$ standard error of mean (SEM). *Indicates $P < .05$ versus control.

Figure 2. Western analysis of matrix metalloproteinase 9 ([MMP-9], left graph) and MMP-13 (right graph) of control (untreated) and nicotine-treated (N = 4) fetal heart ventricles. Separate gels were run for both MMP-9 and MMP-13. Bands corresponding to both pro (92 kDa for MMP-9 and 57 kDa for MMP-13) and active (86 kDa for MMP-9 and 45 kDa for MMP-13) forms were identified. Density values of each band were analyzed relative to either α -actin (for MMP-9) or glyceraldehyde 3-phosphate dehydrogenase ([GAPDH]; for MMP-13). Values are mean $\pm$ standard error of mean (SEM).

treated with nicotine alone. In a separate gel, NAC alone had no effect on MMP-2 protein levels (not shown). Further, based on gel zymography, NAC reduced levels of active MMP-2 but had no significant effect on pro MMP-2 levels (Figure 3).

Collagen Staining by Immunohistochemistry

Matrix metalloproteinases are important enzymes in cardiac remodeling²⁵ regulating the protein expression of the ECM.

Picrosirius red staining was used to identify interstitial collagen fibers within fetal heart ventricles of control, nicotine-, NAC alone-, and nicotine + NAC-treated animals (Figure 4). Under conventional light microscopy, fetal heart tissue exhibited a collagenous network of fibers (red) surrounding muscle bundles (yellow) within the ventricle along with red staining of collagen on the epicardial surface. Prenatal nicotine treatment increased interstitial collagen fibers distributed within the myocardial interstitium between cardiomyocytes.

Figure 3. The effect of *N*-acetylcysteine (NAC) on fetal cardiac matrix metalloproteinase 2 (MMP-2) expression of nicotine-treated animals by Western analysis (top left) and gelatin zymography (right). Negative controls (no nicotine) of NAC alone on MMP-2 expression in fetal hearts is shown in the bottom left graph. Matrix metalloproteinase 2 bands were identified in Western immunoblots at ~72 kDa and normalized to bands corresponding to α-actin as a loading control. Gel zymography identified both pro (72 kDa) and active (62 kDa) bands for MMP-2. Band densities for gel zymography were quantified by densitometry and expressed as arbitrary values. Values are mean ± standard error of mean (SEM). *Indicates $P < .05$ versus control.

Figure 4. Immunostaining of interstitial collagen of fetal heart ventricles by Picrosirius red stain. Interstitial collagen of hearts of age-matched fetal guinea pigs ($N = 3$ for each group) was identified by Picrosirius stain in control (top left), nicotine (top right), and in the presence (+) and absence (−) of *N*-acetylcysteine (NAC). Interstitial collagen fibers are identified as red stain (black arrows) in cardiac muscle. Magnification = $\times 200$. Bottom graph illustrates the mean density values per area ($\times 1000$) of Picrosirius red stain of fetal guinea pig hearts quantified by NIH Image J software. *Indicates a significance difference ($P < .05$) from C (control), NAC, and NIC (nicotine) +NAC; + indicates a significance difference from NAC alone and untreated control (C).

In nicotine-treated hearts, collagen fibers (arrows) appeared elongated and fibrous, compared to sparse, compact, and shortened in normoxic control hearts (top graph, top row). In the presence of NAC, the amount of interstitial collagen fibers was reduced and their appearance resembled that of the untreated controls (top graph, bottom row). Quantification of positive staining was performed and illustrated in Figure 4 (graph). There was a significant increase in red stain in the presence of nicotine compared to untreated controls. In the presence of both nicotine and NAC, collagen fibers were similar in both staining density and morphological characteristics compared to untreated controls and NAC alone.

Discussion

This study demonstrates that prenatal nicotine increases active MMP-2 protein expression and interstitial collagen in left ventricles of fetal guinea pig hearts. In contrast, there were no changes in either MMP-9 or -13 levels in response to nicotine exposure. Treatment with the antioxidant, NAC, inhibited both the nicotine-induced increase in MMP-2 levels and collagen staining but had no effect alone. Thus, prenatal nicotine targets the fetal heart and the expression of selected MMPs important in cardiac remodeling via oxidative stress. Further study is needed to determine whether consequences of nicotine exposure in the fetal heart may have lasting effects that could potentially disrupt normal cardiac morphology and function postnatally.

Role of MMPs in Cardiac Remodeling

Cardiac MMPs play an important role in regulating the structural integrity of the ECM.^{26,37} Altered MMP expression and activation can lead to an imbalance between collagen synthesis/degradation in the myocardial ECM^{37,38} and a change in the structural integrity of the heart.^{26,39} In the adult heart, the role of MMPs is associated with repair and protection in response to pathological injury³⁹; while in the immature heart, MMPs play an essential role in tissue remodeling and cell migration.²⁷ Growth of the fetal heart occurs by both hyperplasia and hypertrophy of cardiomyocytes⁴⁰ and structural integrity of the myocardium is regulated by the complex balance of synthesis/degradation of ECM components such as fibrillar collagens. The effect of nicotine on the expression levels of selected MMPs in the myocardium may contribute to an alteration in the normal growth processes of the fetal heart leading to cardiomyopathy.⁴¹

Mechanism of Nicotine-Induced MMP Activation

Prenatal nicotine exhibited a selective effect on MMP-2 expression in the fetal guinea pig myocardium. Matrix metalloproteinases 2, 9, and 13 are important proteolytic enzymes in cardiac ventricles and in remodeling of the myocardium.²⁸⁻³⁰ Cardiac remodeling can occur as a result of either altered expression of MMP subtypes or by factors that alter local

activation of selected MMPs.⁴² Our study showed that prenatal nicotine exposure increased fetal cardiac MMP-2 protein levels associated with the active form only but did not alter either pro or active MMP-9 or -13 levels. Further, the nicotine-induced increase in active MMP-2 was reversed by NAC, suggesting that posttranslational modification rather than transcriptional regulation is altered by nicotine exposure.

The mechanism by which nicotine activates MMPs in the fetal myocardium is complex and poorly understood. Nicotine has been reported to increase catecholamine release² and ROS generation,⁴³⁻⁴⁵ both of which could contribute to MMP activation. Nicotine increases catecholamine levels in the fetal circulation² by increasing neurotransmitter release from sympathetic nerve fibers and activation of the adrenal medulla.⁹ Circulating substances such as norepinephrine can activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a superoxide-generating enzyme, via receptor-mediated protein kinase C activation.⁴⁶ Nicotine may also increase ROS generation in the myocardium by enhancing neutrophil and macrophage invasion⁴⁵ via activation of the phagocytic NADPH oxidase^{23,47} in nicotine-stimulated immune cells, contributing to increased oxidative stress. Reactive oxygen species (eg, superoxide anion and hydrogen peroxide) have been shown to be important regulatory factors in the activation of MMPs.^{25,26} For example, pro-MMP can be activated to the active form by ROS via autolytic cleavage of the cysteine thiol group,^{25,48} independent of protein expression. Although ROS generation was not measured, the inhibitory effect of NAC on both nicotine-induced MMP-2 activity and collagen accumulation suggests evidence of oxidative stress. We are unaware of any evidence that NAC can directly inactivate MMPs. Further study is needed to quantify oxidative stress in nicotine-treated fetal hearts.

Nicotine has been reported to constrict the uterine circulation and induce fetal hypoxemia, however, this is dose dependent and variable among species.^{2,49} We do not think nicotine's effects are mediated by an hypoxemia-induced challenge under the conditions of our study. This is based on the lack of an expected hypoxemia-induced increase in relative placental weight (placental wt/fetal body wt ratio) and the lack of a hypoxemia-induced increase in fetal cardiac MMP-9 protein expression, both of which have been measured in the fetuses of hypoxemic guinea pig.⁵⁰ In the same study, MMP-2 protein expression was unaltered by fetal hypoxemia⁵⁰ although increased in the current study when fetal guinea pigs were exposed to nicotine. Therefore, hypoxemic stress, under the current conditions, is unlikely to account for the differential effect on MMP expression in fetal guinea pig hearts.

Role of Collagen in Nicotine-Induced Cardiac Remodeling

Activation of MMP results in the degradation of matrix components of the heart and plays an important role in modulating collagen synthesis.⁴² Following activation, collagen degradation initially exceeds that of collagen synthesis.²⁵ Interstitial collagen fibers are degraded first by collagenases such as

MMP-13 and then by gelatinases such as MMP-2 and -9 into matrikines.⁴² With prolonged MMP activation, these subfragments accumulate and feedback to stimulate further collagen synthesis.^{39,42} As a result, accumulation of interstitial collagen and cross-linking of collagen fibers occurs in a time-dependent manner leading to the generation of cardiac fibrosis.⁴² This study demonstrates that prenatal nicotine contributes to the accumulation of collagen by the activation of MMP-2 in the fetal myocardium, both of which are inhibited by NAC. While MMP-13 activity was not increased with nicotine exposure, the active form was in high abundance, which may contribute significantly to substrate generation for downstream MMPs such as MMP-2. The mechanism mediating nicotine-induced increase in myocardial collagen is complex and is likely mediated by the interaction between the relative activities of the different MMP subtypes, with MMP-2 playing an important role in the fetal guinea pig heart.

Summary

Cigarette smoke contains thousands of toxic components that can impact the health and well-being of the fetus. Nicotine is one of the most biologically active and toxic components of tobacco smoke² and can readily cross the placenta. The effect of nicotine on fetal hearts differs from that on adult hearts because fetal heart growth is a transitional process of terminal differentiation and cardiac remodeling via apoptosis and synthesis/degradation of ECM components. Disruption of either functional or structural components during critical stages of growth and development is expected to have lasting effects, postnatally. Thus, the altered regulation of MMP expression by prenatal nicotine identifies an important pathway that may increase the susceptibility of the fetus to cardiac dysfunction. This is the first study to show that prenatal nicotine increases MMP-2 protein levels in intact fetal guinea pig hearts and may be initiated by oxidative stress. Further study is needed to identify the specific molecular mechanisms by which nicotine cytotoxicity occurs in fetal organs in order to develop therapies that can be targeted for prevention of both immediate and long-term effects in the postnatal heart.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: supported in part by NIH RO1 HL49999 (LPT), NIH RO1 HL85037 (JAM), F31 HL090044 (LE) from the National Heart, Lung and Blood Institute and the University of Maryland Statewide Health Network/Other Tobacco-Related Diseases Grant.

References

1. Substance Abuse and Mental Health Services Administration. *Results from the 2004 National Survey on Drug Use and Health: National Findings, Tobacco Use (PDF -1.17MB)*. Rockville, MD: Substance Abuse and Mental Health Services Administration, Office of Applied Studies; 2005.
2. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. *Semin Perinatol*. 1996;20(2):115-126.
3. Slotkin TA. If nicotine is a developmental neurotoxicant in animal studies, dare we recommend nicotine replacement therapy in pregnant women and adolescents? *Neurotoxicol Teratol*. 2008;30(1):1-19.
4. Duncan JR, Randall LL, Belliveau RA, et al. The effect of maternal smoking and drinking during pregnancy upon (3)H-nicotine receptor brainstem binding in infants dying of the sudden infant death syndrome: initial observations in a high risk population. *Brain Pathol*. 2008;18(1):21-31.
5. Slotkin TA. Prenatal exposure to nicotine: what can we learn from animal models? In: Zagon IS, Slotkin TA, eds. *Maternal Substance Abuse and The Developing Nervous System*. San Diego, CA: Academic Press; 1992:97-124.
6. Changeux J-P. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nature Rev Neurosci*. 2010;11(6):389-401.
7. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Rev Cancer*. 2003;3(10):733-744.
8. Eggleton RD, Brown KC, Dasgupta P. Nicotine acetylcholine receptors in cancer: multiple roles in proliferation and inhibition of apoptosis. *Trends Pharmacol Sci*. 2008;29(3):151-158.
9. Haass M, Kubler W. Nicotine and sympathetic neurotransmission. *Cardiovasc Drugs Ther*. 1996;10(6):657-665.
10. Hanna ST. Nicotine effect on cardiovascular system and ion channels. *J Cardiovasc Pharmacol*. 2006;47(3):348-358.
11. Wang H, Shi H, Zhang L, et al. Nicotine is a potent blocker of the cardiac A-type K⁺ channels. *Circulation*. 2000;102(10):1165-1171.
12. Grilli M, Parodi M, Raiteri M, Marchi M. Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release. *J Neurochem*. 2005;93(5):1353-1360.
13. Jacobs I, Anderson DJ, Surowy CS, Puttfarcken PS. Differential regulate of nicotine receptor-mediated neurotransmitter release following chronic (-)-nicotine administration. *Neuropharmacology*. 2002;43(5):847-856.
14. Levin ED, Lawrence S, Petro A, Horton K, Seidler FJ, Slotkin TA. Increased nicotine self-administration following prenatal exposure in female rats. *Pharmacol Biochem Behav*. 2006;85(3):669-674.
15. Maritz GS, Morley CJ, Harding R. Early developmental origins of impaired lung structure and function. *Early Hum Dev*. 2005;81(9):763-771.
16. Xiao D, Huang X, Lawrence J, Yang S, Zhang L. Fetal and neonatal nicotine exposure differentially regulates vascular contractility in adult male and female offspring. *J Pharm Exp Ther*. 2007;320(2):654-661.
17. Zhang S, Day I, Ye S. Nicotine induced changes in gene expression by human coronary artery endothelial cells. *Atherosclerosis*. 2001;154(2):277-283.
18. Lawrence J, Xiao D, Xue Q, Rejali M, Yang S, Zhang L. Prenatal nicotine exposure increases heart susceptibility to

- ischemia/reperfusion injury in adult offspring. *J Pharmacol Exp Ther*. 2008;324(1):331-341.
19. Lawrence J, Chen M, Xiong F, et al. Foetal nicotine exposure causes PKC1 gene repression by promoter methylation in rat hearts. *Cardiovasc Res*. 2011;89(1):89-97.
20. Barros DM, Galhardi FG, Ferreira JLR, et al. The benefits and drawbacks of nicotine exposure in the cortex and hippocampus of old rats. *Neuro Toxicol*. 2007;28(3):562-568.
21. Bruin JE, Petre MA, Lehman MA, et al. Maternal nicotine exposure increases oxidative stress in the offspring. *Free Radic Biol Med*. 2008;44(11):1919-1925.
22. Cormier A, Morin C, Zini R, Tillement J-P, Lagrue G. In vitro effects of nicotine on mitochondrial respiration and superoxide anion generation. *Brain Res*. 2001;900(1):72-79.
23. Fang Q, Sun H, Arrick DM, Mayhan WG. Inhibition of NADPH oxidase improves impaired reactivity of pial arterioles during chronic exposure to nicotine. *J Appl Physiol*. 2006;100(2):631-636.
24. Jaimes EA, Tian R-X, Raji L. Nicotine: the link between cigarette smoking and the progression of renal injury? *Am J Physiol Heart Circ Physiol*. 2007;292(1):H76-H82.
25. Deschamps AM, Spinale FG. Pathways of matrix metalloproteinase induction in heart failure: bioactive molecules and transcriptional regulation. *Cardiovasc Res*. 2006;69(3):666-676.
26. Rutschow S, Li J, Schultheiss H-P, Pauschinger M. Myocardial proteases and matrix remodeling in inflammatory heart disease. *Cardiovasc Res*. 2006;69(3):646-656.
27. Chow AK, Cena J, Schulz R. Acute actions and novel targets of matrix metalloproteinase in the heart and vasculature. *Br J Pharmacol*. 2007;152(2):189-205.
28. Benowitz NL, Gourlay SG. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. *J Am Coll Cardiol*. 1997;29(7):1422-1431.
29. Jauniaux E, Glubis B, Acharaya G, Thiry P, Rodieck C. Maternal tobacco exposure and cotinine levels in fetal fluids in the first half of pregnancy. *Obstet Gynecol*. 1999;93(1):25-29.
30. Beloosesky R, Gayle DA, Amidi F, et al. N-acetyl-cysteine suppresses amniotic fluid and placenta inflammatory cytokine responses to lipopolysaccharide in rats. *Am J Obstet Gynecol*. 2006;194(1):268-273.
31. Demiralay R, Gursan N, Erdem H. The effects of erdosteine, N-acetylcysteine and vitamin E on nicotine-induced apoptosis of cardiac cells. *J Appl Toxicol*. 2007;27(3):47-54.
32. Langley SC, Kelly FJ. N-acetylcysteine ameliorates hyperoxic lung injury in the preterm guinea pig. *Biochem Pharmacol*. 1993;45(4):841-846.
33. Ferrari R, Ceconi C, Curello S, et al. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med*. 1991;91(3C):95S-105S.
34. Wilkes JM, Clark LE, Herrera JL. Acetaminophen overdose in pregnancy. *South Med J*. 2005;98(11):1118-1122.
35. Oh C, Dong Y, Liu H, Thompson LP. Intrauterine hypoxia upregulates proinflammatory cytokines and matrix metalloproteinases in fetal guinea pig hearts. *Am J Obstet Gynecol*. 2008;199(1):78.e1-78.e 6. Epub 2008.
36. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue. *Histochem J*. 1979;11(4):446-455.
37. Nagase H, Woessner JF. Matrix metalloproteinases. *J Biol Chem*. 1999;274(31):21491-21494.
38. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res*. 2004;94(12):1543-1553.
39. Lopez B, Gonzalez A, Diez J. Role of matrix metalloproteinase in hypertension-associated cardiac fibrosis. *Curr Opin Nephrol Hypertens*. 2004;13(2):197-204.
40. Clubb FJ, Bishop SP. Formation of binucleated myocardial cells in the neonatal rat. An index for growth hypertrophy. *Lab Invest*. 1984;50(5):571-577.
41. Baykan A, Narin N, Narin F, Akgun H, Yavasacn S, Saraymen R. The protective effect of melatonin on nicotine-induced myocardial injury in newborn rats whose mothers received nicotine. *Anadolu Kardiyol Derg*. 2008;8(4):243-248.
42. Li YY, McTiernan CF, Feldman AM. Interplay of matrix metalloproteinases, tissue inhibitors of metalloproteinases and their regulators in cardiac matrix remodeling. *Cardiovasc Res*. 2000;46(2):214-224.
43. Newman MB, Arendash GW, Shytle RD, Bickford PC, Tighe T, Sanberg PR. Nicotine's oxidative and antioxidant properties in CNS. *Life Sci*. 2002;71(24):2807-2820.
44. Zhou X, Sheng Y, Yang R, Kong X. Nicotine promotes cardiomyocyte apoptosis via oxidative stress and altered apoptosis-related gene expression. *Cardiology*. 2010;115(4):243-250.
45. Owasoyo JO, Jay M, Gillespie MN. Impact of nicotine on myocardial neutrophil uptake. *Toxicol Appl Pharmacol*. 1998;92(1):86-94.
46. Akki A, Zhang M, Murdoch C, Brewer A, Shah AM. NADPH oxidase signaling and cardiac myocyte function. *J Mol Cell Cardiol*. 2009;47(1):15-22.
47. Yildiz D, Liu YS, Ercal N, Armstrong DW. Comparison of pure nicotine- and smokeless tobacco extract-induced toxicities an oxidative stress. *Arch Environ Contam Toxicol*. 1999;37(4):434-439.
48. Ra H-J, Parks WC. Control of matrix metalloproteinase catalytic activity. *Matrix Biol*. 2007;26(8):587-596.
49. Luck W, Nau H, Hansen R, Steldinger R. Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. *Dev Pharmacol Ther*. 1985;8(6):384-395.
50. Thompson L, Dong Y, Evans L. Chronic hypoxia increases inducible NOS-derived nitric oxide in fetal guinea pig hearts. *Pediatr Res*. 2009;65(2):188-192.