Influence of Prenatal Administration of Nicotine/Thiocyanate on the Morphology of Exocrine Pancreas of 1–Month-Old Rat Offspring

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Background: Prenatal exposure to cigarette smoking is associated with harmful effects which might result in impaired pancreatic functions in the offspring. Cigarettes contain many ingredients, such as nicotine (NC) and thiocyanate (TC). NC has been proven to have variable hazardous effects. The exocrine pancreas has been found to be affected by NC. This effect might be related to the activation of pancreatic stellate cells (PSC).

Objective: To evaluate the effects of nicotine/thiocyanate on the exocrine pancreas of developing rats.

Design: An Experimental Animal Study.

Setting: Animal House, Faculty of Medicine, Assiut University, Egypt.

Method: Fifteen pregnant female rats were divided into three groups: group I (control), group II (nicotine-treated NC), and group III (thiocyanate-treated TC). Both male and female offspring of each group were sacrificed 1 month postnatal. The exocrine pancreatic tissues were processed for light microscopic evaluation and immunohistochemical detection of α-smooth muscle actin.

Result: In the NC-treated group, there was an increase in the spaces between the acini and vacuolation of the cytoplasm and darkening of the nuclei. There was an increase in collagen fibers in the NC-treated group. Immunohistochemical evaluation revealed an increase in the staining of α-smooth muscle actin (marker of PSC). On the other hand, the TC-treated group revealed minimal histomorphological effect, no increase in collagen fibers, as well as minimal α-SMA-immunostaining.

Conclusion: The prenatal nicotine administration resulted in marked structural changes, fibrosis as well as activation of PSC. Thiocyanate treatment resulted in minimal changes. This highlights the role of nicotine as the major player in cigarette smoking, especially during pregnancy.

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Cigarette smoking is a risk factor for many diseases, affecting several organs including the pancreas[1,2,3]. It is well known that tobacco contains many compounds that are toxic to cells and tissues[4,5]. The most important ingredient in tobacco, which is linked to disease pathophysiology, is nicotine (NC). It is a known addictive substance and also considered carcinogenic[6,7]. In pregnant females who smoke, NC crosses the placenta and concentrates in fetal blood and amniotic fluid and could be detected in breast milk during lactation[8,9,10].

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It has been proposed that the pathophysiological changes in the pancreas of a smoker, whether acute or chronic pancreatitis, are linked primarily to NC[7,11]. Studies showed that animals exposed to NC developed changes in pancreatic function and structure[12]. In addition, it was reported that NC treatment resulted in acinar cell damage, independent of the method of application, either orally or via inhalation[1]. Myofibroblasts or pancreatic stellate cells (PSC) produce some factors that mediate replication or replacement of lost or damaged cells. Chronic diseases, such as pancreatitis are accompanied by activation of these stellate cells[2,13]. The activated stellate cells secrete α-SMA and collagens and inflammatory cytokines and growth factors, which may play a role in the repair of injured pancreas[9,14].

Another component in cigarette smoke is thiocyanate (TC), which is present universally in the extracellular fluid of mammals, such as saliva, plasma, milk, tears, and gastric fluid. Plasma values of TC are much higher in smokers compared to non-smokers; therefore, it is used to verify the level of exposure to tobacco smoke and as a biomarker of quitting smoking[15]. TC accesses the body via ingestion of some cyanogen-rich plants, such as cassava, yam, maize, and linseed[16,17]. Furthermore, it can be formed as a detoxification product of cyanide, another component of tobacco, by certain mitochondrial enzymes[18]. Although TC is being associated with cyanide, it is considered to be somewhat non-toxic at certain concentrations[18]. Therefore, there are two main groups at increased risk of maximum exposure to TC: a group that consumes a diet rich in cyanogenic plants and a group of smokers[15,19]. The effects of smoking are difficult to study due to multiple variables of toxicity caused by tobacco smoke and the presence of many components in tobacco[7,20]. Therefore, it is important to highlight the significance of each component of cigarette that has adverse effects on the pancreas. Several studies have addressed the effects of NC or TC on adult exocrine pancreas in animal models. In addition, few studies reported prenatal exposure of NC and TC on other organs as the thyroid or adrenal and few have studied the endocrine pancreas[5,9,21]. To our knowledge, no study addressed the prenatal effects of NC and TC on the exocrine pancreas morphology.

The aim of this study is to evaluate and compare the effects of nicotine, as well as thiocyanate on the exocrine pancreatic histology of 1-month-old offsprings of pregnant rat mothers.

METHOD

Fifteen 5-month-old, pregnant albino Wistar rats were used in this study. They were kept in the animal house under appropriate conditions with free access to food and water. The pregnant rats were divided into three groups. Group I: is the control group. Group II: the rats were given nicotine (6 mg/kg/day) by subcutaneous route from gestation day 4–20. Group III: thiocyanate-treated group were treated with oral potassium thiocyanate (25 mg/kg/day) from gestation day 4–20[21]. Six offsprings (males and females) from each group were sacrificed at the age of 1 month and the heads of the pancreas were obtained for the study. Paraffin sections (5 µm) were obtained. Masson›s trichrome stain was used[22].

Immunostaining of acinar cells was performed on paraffin sections. Sections (7 µm) were deparaffinized and rehydrated and processed using the universal kit (ultravision detection system, anti-polyvalent, HRP/DAB, Thermo Fischer Scientific, Fremont, CA, USA). The sections were boiled in the microwave in 10 mM citrate buffer at pH 6.0 for 20 minutes then cooled at room temp for 20 minutes. The immunohistochemical reaction was demonstrated using streptavidin-biotin immunoperoxidase method. The primary antibody, anti-α-smooth muscle actin (mouse monoclonal antibody, CGA7, Cat. # sc-53015, Santa Cruz biotechnology inc., Dallas, Tx, USA) was used at 1:500 dilution. The primary antibody was applied for 30 minutes. The sections were incubated with the secondary antibody (biotinylated goat anti-polyvalent) for 10 minutes. The reaction was then visualized using diaminobenzidine for 10 min. The sections were counterstained with Mayer's hematoxylin. Examination of light microscopic slides and photographs using a light microscope connected to a camera.

Morphometry was performed by using a computerized image analyzer system software (Axio Scope A1, Carl Zeiss Microscopy, Germany). The surface area of the pancreatic acini was measured in square micrometers. Five non-overlapping fields in ten serial sections of the pancreatic acini of three different rats of the three studied groups were included in the measurement. Measurements of the surface area of the pancreatic acini were done in H&E-stained sections using 40X magnification lens. The t-test was used to compare the mean differences of the surface area of the pancreatic acini of the three studied groups. The P-value of less than 0.05 was considered statistically significant.

RESULT

Group 1 (control) histological examination revealed normal morphology of the pancreas. It is formed of round to oval pancreatic acini with a small lumen. These acini were separated by very little connective tissue septa. Ducts were present in between the acini. The acinar cells had the characteristic basal basophilia and apical acidophilia. The nuclei appeared vesicular, rounded and basal, see figure 1 (A).

Examination of nicotine-treated animals (group II) the specimens had marked degenerative changes. Most of the specimens were affected and revealed focal lesions of variable degrees of cellular degeneration, such as vacuolations and dark or pyknotic nuclei. The acini were separated by wide spaces that may be due to interstitial edema and increased interlobular connective tissue. Degenerative changes of the acini were focal leaving empty spaces which resulted in disturbance of the normal architecture of the pancreas, see figure 1 (B).

Group III (thiocyanate treated), the specimens revealed minimal degenerative changes and the pancreatic architecture was preserved. There were moderate spaces between the acini. Most acinar cells and their nuclei were not affected, see figure 1 (C).

Masson's trichrome-stained sections of the pancreas of group I revealed no collagen fibers between acini, ducts; only found around the blood vessels, see figure 2 (A). However, sections of the pancreas of group II (nicotine treated) displayed large amounts of bundles of collagen fibers, particularly around the acini and ducts, see figure 2 (B). Sections of the pancreas of

group III (thiocyanate treated) revealed few collagen fibers around the blood vessels, see figure 2 (C).

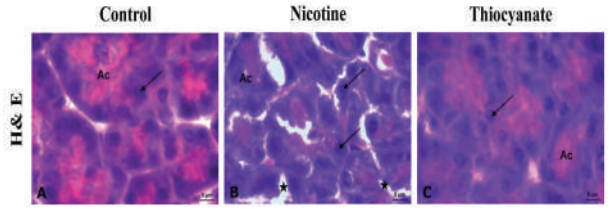

Figure 1 (A-C): Morphology of the Acini of the Pancreas in H&E Stained Sections. (A) Group I: Showing Normal Structure of the Pancreas. The Acini (AC) Inside the Lobules, The Cells Appear Pyramidal in Shape with Basal Basophilia and Apical Acidophilia. Note Vesicular Nuclei (arrow). (B) Group II: Showing the Disturbed Structure of the Acini of this Group. Marked Increase in Connective Tissue Septa as Revealed by Marked Empty Spaces (Stars). Note the Degenerated Cells with Dark Nuclei (Arrows) in the acini. (C) Group III Showing Undisrupted Morphology with Intact Acini with Moderate Spaces between Acini. Note the Pale Nuclei (Arrow). H& E X 1000

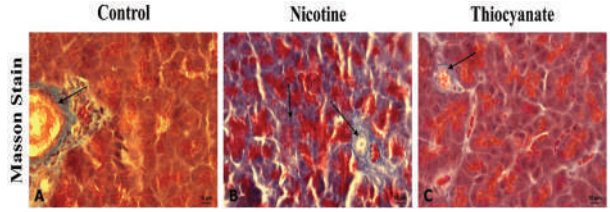

Figure 2 (A-C): Show Sections of the Pancreas Stained with Masson Trichrome Stain for Collagen Fibers. (A) Group I: Showing Negative Staining Around the Acini and the Blue Stained Collagen Fibers Only Surrounding the Blood Vessels (Arrow). (B) Group II: Showing Excess Blue Stained Coarse Collagen Fibers in the Connective Tissues Surrounding the Acini and Ducts (Arrows). (C) Group III Appears with Minimal Collagen Fibers, Which Can Be Seen Around Blood Vessels (Arrow). Masson Trichrome X 400

Immunostainining with α-smooth muscle actin (α-SMA), the control group revealed a negative reaction in the acini, the staining was only confined to blood vessels, see figure 3 (A). The α-SMA immunohistochemical-stained sections of the nicotine-treated group revealed intense cytoplasmic reaction in many acini, such as brown coloration especially at the periphery of the acini, which was more than the control group, see figure 3 (B). The immunohistochemical-stained sections of the thiocyanate group showed that most of the acini revealed decreased α-SMA expression, see figure 3 (C).

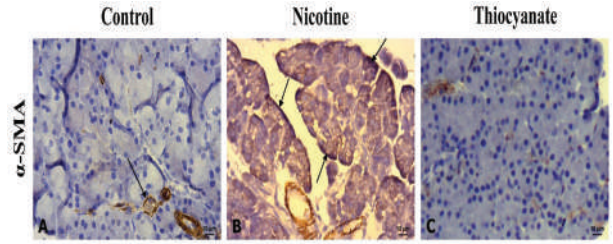

Figure 3 (A-C): Show Sections of Pancreas Immunostained with α-Smooth Muscle Actin. (A) Group I: Showing Negative Staining of the Acini. The Positive Staining is Only around Blood Vessels (Arrow). (B) Group II: Showing Positive Staining (Arrows) Especially at the Periphery of the Acini Indicating Activation of Pancreatic Stellate Cells. (C) Group III: Showing Very Mild Staining of Few Cells. α-SMA Immunostaining X 400.

Table 1: Comparison between the Mean Surface Area of Pancreatic Acini

	Control (n=100)	Nicotine (n=100)	Thiocyanate (n=100)
Mean Surface Area of Pancreatic Acini (μm²)	544.51±66.93	237.41±34.78	449.59±58.53
t-test		40.713	10.675
95% C.I		(292.19, 322.00)	(77.38, 112.45)

The mean surface area of the pancreatic acini in the nicotine and the thiocyanate group were significantly decreased compared to the control group. This decrease was much more remarkable in the nicotine-treated group, see table 1 and figure 4.

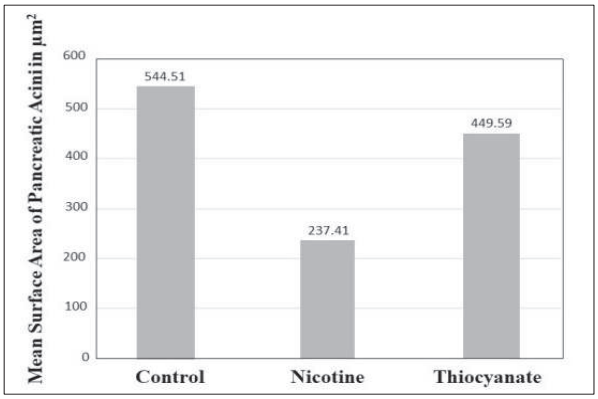

Figure 4: Shows the Graphic Representation of the Surface Area of Pancreatic Acini of the Three Groups. Marked Decrease in Surface Area in Nicotine-Treated Group. The Thiocyanate Group Also Showed a Decrease in Surface Area but Not as That of the Nicotine Group

DISCUSSION

In this study, nicotine resulted in histological changes in the pancreatic acini (exocrine pancreas); degenerative changes were focal. These changes were in the form of cytoplasmic vacuolization of acinar cells. In addition, there was a disruption of the pancreatic architecture resulting from an increase in the connective tissue or due to edema. Similar histological findings were reported in the pancreas by other investigators[7,11,23]. This study revealed a decrease in the tissue of the pancreatic acini in the nicotine-treated group compared to controls. This finding was confirmed by the morphometric results which revealed a decrease in the surface area of the acini in the nicotine group, which was highly significant. On the other hand, the thiocyanate group also showed a decrease in the acinar surface area, but much less remarkable than the nicotine group. Similar results were reported in previous studies where a decrease in surface area of pancreatic acini was reported in orlistat-treated rats[24].

In this study, there was an increase in connective tissue fibers (collagen) in the nicotine-treated group, which was seen as a big interlobular septa in H&E sections. The result was verified by an increase in Masson trichrome staining around the acini, ducts and blood vessels, which showed large amounts of collagen fibers. The resulting fibrosis may have been produced

by pancreatic stellate cells. Our study revealed marked activation of PSC in the nicotine-treated group, as shown by α-SMA immunostaining. These cells were found to play a major role in the development of pancreatic fibrosis[2,25,26]. If activated, these cells acquire a morphology similar to fibroblasts, become able to synthesize collagens and fibronectins, and migrate from perivascular to periacinar areas[27,28]. The formation of collagen by stellate cells is stimulated by oxidative stress or by the production of a wide variety of cytokines or pro-inflammatory mediators as IL1, IL6[25,27,29]. Therefore, it could be postulated that the interstitial fibrosis was very important in the disorganization of the morphology of the pancreatic acini, which was observed in our study.

Our study demonstrated that fetal nicotine exposure resulted in marked degenerative changes to the pancreatic acini in the offspring of nicotine-exposed mothers, which affects the function. These changes progressively worsen with age even though nicotine exposure was discontinued a little before birth. These results highlight some deleterious health consequences to the offspring due to cigarette smoking during pregnancy and lactation.

Several studies reported that nicotine resulted in disturbances in the synthesis of digestive enzymes and change in their secretion pattern eventually leading to damage to acinar cells[7,11]. The mechanism underlying the cellular damage due to nicotine administration is not yet fully understood[30]. In the case of the pancreatic damage due to cigarette smoking, precipitates of protein secretion are formed; these are released in smaller pancreatic ducts and obstruct the drainage of pancreatic juice[31,32].

In this study, the thiocyanate group revealed that the pancreas had minimal effect compared to the nicotine group. Thiocyanate is considered to be a metabolite of cyanide[17,33]. Cyanide is transformed through a mitochondrial enzyme to thiocyanate that is excreted mainly through the urine[34]. Cyanide toxicity is also manifested by increased levels of plasma thiocyanate[18,35]. Chronic cyanide toxicity may explain the occurrence of pancreatitis in patients who consume cyanogenic compounds especially cassava[36,37]. In addition, the toxicity was also linked to patients who consume alcoholic beverages, and smoke cigarettes simultaneously[38].

CONCLUSION

This study revealed some morphological changes to the exocrine pancreas due to prenatal administration of nicotine and thiocyanate. These changes were more pronounced with nicotine rather than thiocyanate. The evidence from our study suggests a direct relation between cigarette smoking and pancreatic damage, which is shown to be a multifactorial process. To unravel the mechanisms underlying cigarette smoke-induced pancreatic damage, future studies are needed to evaluate the pathophysiology of the effect of nicotine during pregnancy.

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Competing Interest: None.

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