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ARTICLE

Histopathological Evaluation of Renal Vascular Changes in Rats Exposed to Passive Smoking

Nil CULHACI, Ibrahim METEOGLU, Mehmet DUNDAR,[1] Izzet KOCAK[1]

Departments of Pathology and [1]Urology, Adnan Menderes University, Faculty of Medicine, Aydin, Turkey

Cigarette smoking is an important risk factor for renal damage due to its effects on small interlobular arteries. We investigated the effects of long-term passive smoking on renal vascular structures in healthy rats exposed to smoke soon after birth. Forty-two Sprague-Dawley rats (21 males and 21 females) exposed to passive smoking comprised the experimental group and 33 non-exposed rats (17 males and 16 females) comprised the control group. The number of renal vessels, as well as the level of glomerular capillary sclerosis, hyalinosis of arterioles, and myointimal hyperplasia of arteries was assessed in renal biopsy specimens. The mean number of renal vessels in male and female rats exposed to passive smoke (21.71 and 13.81, respectively) did not significantly differ from the mean number of renal vessels in male and female control rats (22.47 and 13.06, respectively) ($p>0.05$). Levels of glomerulosclerosis, hyalinosis, and myointimal hyperplasia also did not differ between the experimental and control groups ($p>0.05$). Histopathologic evidence of renal vascular damage was not found in young rats exposed to passive smoke for 4 months. A longer or higher degree of exposure to cigarette smoke components may be required before such changes manifest, and aging and primary renal disease may play a role. (Pathology Oncology Research Vol 11, No 2, 121–124)

Key words: passive smoking, renal vasculature, rat

Introduction

Cigarette smoking is a major risk factor for vascular diseases and various forms of cancer,[1] and the kidney is an important target organ for smoking-induced damage.[2] Chronic cigarette smoking has important adverse effects on renal function in patients with primary hypertension, primary glomerular diseases and diabetic nephropathy, as well as in those undergoing chronic hemodialysis. It is an independent predictor of albuminuria in patients with primary hypertension and has been related to the onset of microalbuminuria or proteinuria, as well as to chronic renal failure in patients with diabetes; smoking also plays a pathogenetic role in the development of idiopathic nodular glomerulosclerosis.[2-5] Kasiske and Klinger reported that smoking more than 25 pack-years was associated with a higher rate of graft loss in renal graft recipients.[6] However, few studies have investigated the effects of passive smoking on renal biopsy tissue in subjects without renal disease.

Smoking is thought to exert its detrimental effects on the kidney by damaging small interlobular arteries.[7] However, this notion is somewhat controversial. Some investigators have attributed histological changes in vascular structures to aging rather than to smoking. In contrast, Gambaro et al.[8] reported that smoking causes renal dysfunction via the actions of vasoactive mediators and that the dysfunction does not result from aging. In this study, we investigated the effects of passive smoking on renal vascular structures in healthy rats exposed to smoke soon after their birth, so that any observed changes would not be related to aging or the presence of other chronic renal diseases.

Materials and methods

Study groups and smoke exposure protocol

Our institution's ethics committee approved the protocol for this study. Twenty female and 10 male Sprague-Dawley rats were placed in cages, with two females and

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Correspondence: Nil CULHACI, MD, Department of Pathology, Faculty of Medicine, Adnan Menderes University, 09100, Aydin, Turkey; Tel.: +90-256-4441256/227, fax: +90-256-2146495, e-mail: e-mail: nculhaci@hotmail.com, nculhaci@adu.edu.tr

one male in each cage. They were mated, and the pups born were kept with their mothers for 3 weeks. After weaning, rats included in the study were housed in 28x28x16 cm cages and received a special feed (Gebze Best Yem Factory).

Seventy-five healthy rats (38 males and 37 females) were included in the study, and divided into 4 groups. Twenty-one male (group 1) and 21 female (group 3) rats were exposed to passive smoke for 120 days. Seventeen male (group 2) and 16 female (group 4) rats not exposed to smoke were used as controls. Rats in groups 1 and 3 were placed in a unit where they were exposed to increasing amounts of cigarette smoke for 120 minutes a day 5 days a week for 4 months; cigarette brands included Birinci, Tekel, associated with high amounts of nicotine. Smoke entered one side of the unit and an aspirator circulated the air within the unit. Rats in groups 1 and 3 were fed after exposure to the smoke, so that their feed would not smell of smoke.

Histopathologic evaluation

Four months into the study, the rats in all four groups were sacrificed. Their kidneys were then harvested, fixed in 10% neutral buffered formalin, and dissected in a plane perpendicular to the interpolar axis, yielding slices of 1 mm. These tissue slices were then embedded in paraffin. For histologic evaluation, 4-μm-thick sections obtained from the paraffin-embedded tissue were stained with hematoxylin & eosin (H&E) and van-Gieson stain. Two pathologists examined all specimens in a blind manner (i.e., they had no knowledge of the subjects' smoking status).

In each biopsy specimen, the glomeruli, arterioles and interlobular arteries were evaluated. The 50 glomeruli evaluated in each specimen were randomly selected. Any glomerulus with evidence of segmental or complete sclerosis was classified as being affected by glomerular capillary sclerosis. Any biopsy specimen that revealed hyalinosis affecting at least one arteriole, or myointimal hyperplasia of at least one vessel was classified as positive for the presence of hyalinosis or myointimal hyperplasia, respectively. To better visualize the vessels, immunohistochemical staining for anti-CD34 antibody (polyclonal, sc-18917, Santa Cruz Biotechnology, CA, USA) was performed. The vessels were counted in each of the ten separate fields.

Statistical analysis

Statistical analysis was performed using the chi-square with continuity correction and Mann-Whitney tests. Data were analyzed using SPSS for Windows 10.0. A p value <0.05 was considered statistically significant.

Table 1. **Number of renal vessels in male and female experimental and control rats**

	N	*Min*	*Max*	*Mean*	*SD*
Male					
Sm	21	19	26	21.71	1.95
NonS	17	17	31	22.47	3.47
Female					
Sm	21	10	18	13.81	2.38
NonS	16	10	17	13.06	1.69

Counts reflect number of vessels counted in 10 fields; Sm: rats exposed to passive smoking, NonS: rats not exposed to passive smoking (controls); SD: standard deviation; p values are not significant

Results

Seventy-five healthy rats were included in the study, consisting of 42 rats exposed to smoke and 33 rats used as controls. *Table 1* shows the number of vessels counted in renal biopsy specimens from male and female rats in each study group. Vessel counts did not significantly differ between the experimental and control groups. *Table 2* summarizes the results of the histological evaluation of potential glomerulosclerosis, hyalinosis and myointimal hyperplasia in the renal biopsy specimens. There was no statistically significant difference in levels of glomerulosclerosis, hyalinosis, or myointimal hyperplasia between the controls and rats exposed to passive smoke.

Discussion

Cigarette smoking increases the risk for cancer and cardiovascular disease.[1] Smoking is also a major risk factor for the progression of renal disease.[2] It plays a role in the genesis of tumors of the urinary system, and has an adverse effect on renal function.[8] However, little is known about the effects of chronic smoking or passive smoking on renal function in those persons whose kidneys are not affected by a specific renal disease. It has recently been reported that smoking may cause renal dysfunction in subjects without documented renal disease,[4,9] but large studies in healthy subjects are lacking.

The most frequently observed abnormality in smokers is an increase in fibroelastic proliferation and hyaline thickening of the intima of arterioles in organs not in direct contact with the cigarette smoke, including the kidney.[10,11] Passive smoking may also cause endothelial dysfunction.[12] It has been suggested that smoking exerts its detrimental effects on the kidney by damaging small arteries.[7] Yet some investigators have attributed histological changes observed in vascular structures to aging rather than smoking. In reality, both aging and smoking may be responsible for these changes.[10]

Oberai et al. reported that only the coronary and renal arterioles, which are thought to be responsive to changes in vascular tone, become thickened in cigarette smokers.[13] They attributed the thickening of arteriole walls to an increase in the collagen content and hyperplasia of smooth muscle. Although this thickening was significantly greater in heavier smokers, these investigators did not report patient ages. Gambaro et al. have reported that smoking causes renal dysfunction via the effects of vasoactive mediators, and that the dysfunction does not result from aging.[8] In contrast to those from nonsmokers, kidney sections obtained at autopsy from smokers without known renal disease indeed showed evidence of changes in the renal microvasculature. However, these may reflect age-related changes. To control for this variable, we exposed young (P21) rats to passive smoking in the present study, and observed no difference between the experimental and control groups in measures of renal vascular damage.

Table 2. **Prevalence of glomerulosclerosis, hyalinosis, and myointimal hyperplasia in male and female experimental and control rats**

		Glomerulosclerosis		*Hyalinosis*		*Myointimal hyperplasia*	
	n	+	−	+	−	+	−
Male							
Sm	21	2 (9.5%)	19 (90.5%)	1 (4.8%)	20 (95.2%)	–	21 (100%)
NonS	17	–	17 (100%)	1 (5.9%)	16 (94.1%)	–	17 (100%)
Female							
Sm	21	–	21 (100%)	4 (19.0%)	17 (81%)	–	21 (100%)
NonS	16	1 (6.2%)	15 (93.8%)	5 (31.2%)	11 (68.8%)	–	16 (100%)

Sm: rats exposed to passive smoking, NonS: rats not exposed to passive smoking; p values are not significant

Gambaro et al. also stated that the mechanism underlying the effect of smoking on renal function is different from that underlying the age-related effect.[8] They noted that endothelin-1 seems to play a role in smoke-associated renal impairment. More recently, Lhotta et al. demonstrated an association between vascular damage in renal biopsy specimens and the smoking habits of patients with kidney disease.[7] There was a significant correlation between smoking and myointimal hyperplasia of the small arteries, which was present in patients older than 50 years and in men but not in younger patients or in women. Stengel et al. also observed gender-related differences in their study.[14] They reported that smoking appeared to be related to the severity of glomerular nephropathy in an at-risk group of men but not in women. In contrast, male gender was not associated with a higher prevalence of myointimal hyperplasia in the present study.

Lhotta et al. also investigated the effects of smoking on glomerulosclerosis, and found no difference in the prevalence of glomerulosclerosis between nonsmokers and ever-smokers.[7] However, they suggested that the number of glomeruli obtained at biopsy may have been insufficient for assessing glomerulosclerosis. To ensure that we obtain sufficient glomeruli, we included the whole cut of renal material from each rat. We also studied young (P21) healthy rats that were all maintained under the same conditions to control for other confounding factors. Under these conditions, the overall prevalence of glomerulosclerosis was low, and did not significantly differ between the smoke-exposed and control rats. Whereas some studies have linked cigarette smoking with renal dysfunction in men but not in women,[7,15] the prevalence of glomerulosclerosis also did not significantly differ between male and female rats exposed to passive smoke.

Smoking has also been associated with adaptive angiogenesis in some tissues. Pfarrer et al. showed that the increase in placental weight in women who smoked during pregnancy is caused by adaptive angiogenesis (increased capillary density) in placental villi.[6] To investigate whether adaptive angiogenesis occurred in the present study, we counted the number of vessels in renal biopsy specimens. Consistent with our other histopathologic findings, there was no significant difference in the number of vessels between experimental and control animals of the same sex.

In the present study, we found no significant difference in either the number of renal vessels or the level of glomerulosclerosis, hyalinosis, or myointimal hyperplasia between smoke-exposed and control rats. Failure to detect such changes might reflect the relatively short duration of the study, thus, of smoke exposure; probably more time is required before such changes become manifest. In addition to a longer experiment, exposure to higher levels of cigarette smoke components may be useful. Odoni et al. showed that application of an acetone extract of cigarette smoke to the oral mucosa of rats further increased indices of glomerulosclerosis and tubulointerstitial damage.[17] They suggested that this model, in which nicotine is more soluble, is more practical and characterized by better absorption of cigarette smoke components than that provided by smoking machines.

In summary, smoking has well-known adverse effects on the kidneys. We believe that the harmful effects on renal vessel walls induced by smoking, especially passive smoking, are time- and age-related. In addition, the kidneys of those with renal disease are more sensitive to cigarette

smoke-induced injury, as changes such as glomerulosclerosis mainly depend on the presence of a preexisting glomerular inflammatory process.

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