Vasoactive and atherogenic effects of cigarette smoking: a study of monozygotic twins discordant for smoking

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Abstract

The mechanism by which atherosclerotic disease is induced by cigarette smoking has not yet been identified unequivocally. Chronic cigarette smoking and the generation of vasoactive prostanoids and the size of carotid atherosclerotic plaques were studied in nine pairs of identical male twins discordant for smoking for over 20 years. The urinary excretion of 2,3-dinor-thromboxane B_2 (thromboxane B_2 metabolite) of the smoking twin was significantly higher (on average 1·8 times higher) in every pair and that of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (prostacyclin metabolite) was significantly higher (on average 1·3 times higher) in eight of the nine pairs. The ratio of excretion of these metabolites was significantly higher, being 4·0 (95% confidence interval 2·7 to 5·4) among the smokers compared with 2·9 (2·1 to 3·8) among the non-smokers, thus favouring a mechanism of vasoconstriction. Excretion of the thromboxane B_2 metabolite was related to the urinary concentrations of nicotine metabolites. Atherosclerotic plaques detected by ultrasonography in the carotid arteries were significantly larger among smokers but did not correlate with the urinary excretion of prostacyclin and thromboxane B_2 metabolites or intensity of smoking.

Smoking was concluded to induce activation of platelets by an effect mediated by nicotine. The increased prostacyclin production, on the other hand, suggested a compensatory mechanism for the general vasoconstrictive properties of cigarette smoking.

Introduction

Although cigarette smoking is strongly and consistently correlated with the increased incidence of atherosclerotic disease, little information is available about how it exerts its cardiovascular effects.[1] Post-mortem examinations have shown that atherosclerotic lesions are significantly more common in cigarette smokers than non-smokers in the aorta and iliac and cerebral arteries but not consistently so in coronary arteries.[2,3] Studies in animals have suggested that cigarette smoking damages the arterial wall by hypoxia and antigenic mechanisms.[4,5] Nicotine alone carries structural changes in aortic endothelial cells and their intercellular connections, suggesting that their permeability to nicotine is increased.[6]

Smoking is thrombogenic, increasing the risk of complications of atherosclerotic disease, in which arterial thrombosis has an important role.[7-9] The results of studies of dysfunction or hyperreactivity of platelets induced by smoking have not been consistent.[10,11] On the other hand, activation of platelets has been thought to contribute to the genesis of vascular occlusive disease by its role in thrombosis as well as atherogenesis.[12,13]

Smoking also has vasoconstrictive properties,[14] and the effect of the dynamics of the flow of blood locally interacts in atherogenesis and thrombogenesis.[15,16] According to current knowledge, thromboxane A_2, which is the main product of the action of cyclo-oxygenase on arachidonic acid in platelets, acts as a potent vasoconstrictor, and excessive excretion of its metabolites may reflect activation of platelets in vivo. Prostacyclin (prostaglandin I_2), a potent vasodilator, is the predominant product of the same cyclo-oxygenase in vascular endothelial cells and inhibits aggregation of platelets in vitro. The excretion of 2,3-dinor-metabolites of vasoactive prostanoids (thromboxane B_2 derived from platelets and prostacyclin from the vascular endothelium) is thought to reflect the degree of their release locally into the circulation.[17,18]

The aim of the present study was to evaluate the effects of chronic cigarette smoking on the urinary excretion of these vasoactive prostanoids in monozygotic twins who had been discordant for smoking for over 20 years. As atherosclerotic changes influence the concentrations of these vasoactive prostanoids[18] the grade of atherosclerotic plaques was also assessed.

Subjects and methods

We enrolled nine pairs of monozygotic male twins who were discordant for smoking from the Finnish twin cohort, which collected documents of zygosity and history of lifestyle and socioeconomic state by questionnaires in 1975, 1981, and 1986.[19] All the subjects were healthy except one of the smokers, who had undergone a vascular reconstruction for peripheral arterial disease two years before; none was receiving any regular treatment. Their mean age was 40 (range 31-53). Discordance for smoking had lasted on average 22 (range 12-35) years, and mean cigarette consumption among the smokers was 18 (range 5-39) cigarettes daily. The non-smokers had never smoked regularly. To measure the lifelong dose of smoking we calculated so called pack years (which corresponded to smoking one packet of cigarettes daily for a year). We used the colorimetric method of Peach *et al* to measure urinary concentration of nicotine metabolites[20] to confirm the subjects as smokers or non-smokers. The weight and height of each pair of twins were not significantly different, nor were serum concentrations of total cholesterol and low density lipoprotein and high density lipoprotein cholesterol, creatinine clearance, and plasma sodium, potassium, and glucose concentrations, all of which were within normal limits. The mean heart rate of the smokers at rest was slightly higher, but blood pressures did not differ.

The subjects were asked not to take any medicines, especially anti-inflammatory drugs, for two weeks before the study, and alcohol was not permitted for 48 hours before urine was collected. Urine was collected over 12 hours from 2000 to 0800, from which the urinary excretion of cotinine, the prostacyclin metabolite 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, and the thromboxane B_2 metabolite 2,3-dinor-thromboxane B_2 were measured. The results were calculated as absolute values (ng/h) and also corrected for creatinine excretion (pg/mg creatinine). Originally 10 pairs of twins were suitable for the study, but one pair had to be excluded as they failed to follow the given instructions when preparing for the study.

The thromboxane B_2 metabolite was determined by spiking 5 ml of urine with deuterium labelled metabolite at positions 19 and 20, conversion to the methoxime derivative, and extraction on a phenylboronic acid column. The eluate was evaporated to about 1 ml on a rotary evaporator and the

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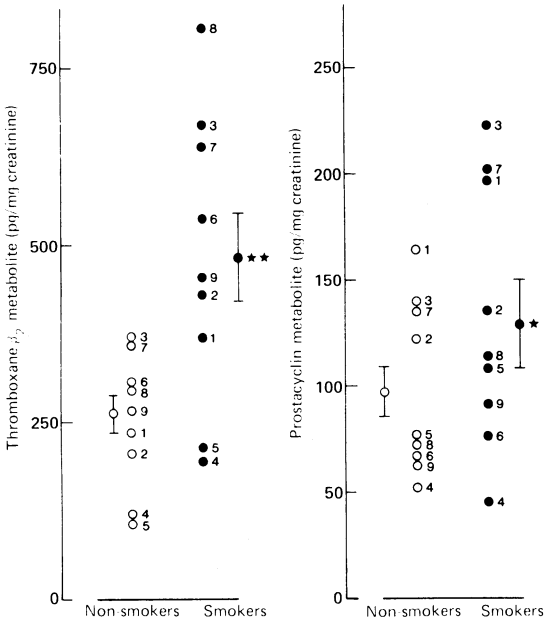
thromboxane derivative alkylated to the pentafluorobenzyl ester during extraction with 1 ml neutral solution of tetrabutylammonium hydrogen sulphate (10 mg/ml, adjusted to pH 7 with sodium hydroxide), 10 µl pentafluorobenzylbromide, and 3 ml dichloromethane. The sample was purified by thin layer chromatography, converted to the trimethylsilylether derivative with bis(trimethylsilyl)trifluoroacetylacetamide, and analysed by gas chromatography-negative ion chemical ionisation mass spectrometry.[21] The prostacyclin metabolite was also assessed by this type of spectrometry.[22]

Atherosclerotic changes of bifurcations of the carotid were assessed by duplex Doppler ultrasonography of the carotid arteries of each subject[23] by a radiologist who did not know which twin was a smoker. An echogenic area was called a plaque if the stenosis it caused was less than 15% of the vessel lumen, otherwise it was termed a stenosis. Breadth and height of the plaques were measured from the transverse section of the ultrasound image of the carotid bifurcation to obtain the transverse area of the plaque.

Statistics—The results in both groups were compared in pairs by determining the 95% confidence intervals for the mean paired differences. Correlation coefficients were calculated to disclose the interrelations between the results in the non-smokers and smokers. Proportional differences were analysed by a paired two tailed *t* test.

Results

Among smokers the number of mean pack years was 14·7 (range 5·2-33·6); the correlation between reported daily smoking and urinary cotinine concentration was not significant (r=0·44). Socioeconomic state and lifestyle were not appreciably different between the smokers and non-smokers, particularly in terms of exercise habits and consumption of alcohol or coffee. Urinary excretion of the thromboxane B_2 metabolite was significantly higher among all nine of the smokers compared with their non-smoking twins, and that of the prostacyclin metabolite was significantly higher in eight of the smokers (p<0·01), whether the results were corrected for creatinine excretion or presented as absolute excretion/hour. The ratio of excreted thromboxane B_2 metabolite to prostacyclin metabolite was significantly higher among eight of the smokers (ratio 4·0 (95% confidence interval 2·7 to 5·4) among smokers *v* 2·9 (95% confidence interval 2·1 to 3·8) among non-smokers; p<0·005). The figure shows the individual results and the table the mean values.



Urinary excretion of thromboxane B_2 and prostacyclin metabolites corrected for creatinine excretion in non-smoking (○) and smoking (●) monozygotic twins (numbered)

Mean urinary cotinine concentration, excretion of prostacyclin and thromboxane B_2 metabolites, and area of atherosclerotic plaques at carotid bifurcation in monozygotic twins discordant for smoking

	Non-smoking twin	Smoking twin	Mean paired difference (95% confidence interval)
Cotinine (mg/l)	0·1	1·4	1·4 (0·5 to 2·2)
Prostacyclin metabolite (µg/g creatinine)	99·7	131·7	21·9 (10·1 to 53·9)
Prostacyclin metabolite (ng/h)	5·9	7·6	1·7 (0·1 to 3·4)
Thromboxane metabolite (µg/g creatinine)	263·2	479·9	216·7 (107·0 to 326·4)
Thromboxane metabolite (ng/h)	16·1	27·9	11·7 (4·7 to 18·7)
Plaque area (mm²)	1·9	6·5	4·6 (0·9 to 8·3)

Changes indicative of atherosclerosis—that is, the areas of echogenic plaques of the carotid arteries—were greater in the smokers. The one smoker who had peripheral arterial disease had the most pronounced lesion of all, a stenosis of 19% in the carotid area. No other subject had any detectable stenosis. The interrelation among the vasoactive metabolites of thromboxane B_2 and prostacyclin, the areas of atherosclerotic plaques, and the intensity of smoking showed significant correlations between urinary concentrations of the thromboxane B_2 metabolite and long term smoking as measured in pack years (r=0·83, p<0·01) as well as short term smoking as measured by urinary cotinine excretion (r=0·67, p<0·05); all other correlations were non-significant.

Discussion

The subjects, monozygotic pairs of male twins whose only apparent discordant factor was smoking, offered a unique opportunity to evaluate the effects of smoking on cardiovascular pathogenesis. As the genetic effects are controlled for, differences in metabolism in each pair can be interpreted as being due to cigarette smoking. The discrepancy noted between the reported number of cigarettes smoked daily and cotinine concentration could have two possible explanations: subjective reports of smoking are unreliable,[24] and the constituents of tobacco smoke are inhaled and absorbed differently in different people.[25] This may cause bias in epidemiological studies, which evaluate the effects of amount of smoking based only on subjective reports.

Our results show that excretion of the thromboxane B_2 metabolite is increased significantly in chronic cigarette smokers, confirming earlier findings.[26-28] The same method was used in our study—that is, measurement of a dinor metabolite from urine—which suggests extrarenal formation of thromboxane in vivo, derived largely from platelets under certain physical conditions.[17] Nowak *et al*, treating subjects with low dose aspirin, showed that platelets are the predominant source of the increase of this metabolite induced by smoking.[27] The fact that among smokers the concentration of the thromboxane B_2 metabolite and the intensity of smoking were highly correlated suggests a direct influence of smoking on platelets. This is also supported by a decline in the thromboxane B_2 metabolite as early as one week after smoking was stopped and an increase again after it was restarted.[26] Also the excretion of the prostacyclin metabolite was enhanced in the twins who smoked. Aortic tissue of rats exposed to cigarette smoke in vitro has, however, a reduced ability to generate prostacyclin.[29]

Similarly, nicotine inhibits prostacyclin synthesis by venous tissue in humans.[30] Our results, which agree with those of Nowak *et al*,[27] do not necessarily conflict with these experiments, in which the ability to generate prostacyclin has been measured in response to strong direct stimuli in vitro as this does not relate to the actual rates of production in vivo.[18]

The degree to which excretion of the thromboxane B_2 metabolite in proportion to that of the prostacyclin metabolite was raised may point to an additional vasoconstrictive load for smokers, among whom sympathoadrenergic activation already suggests enhanced vasoconstriction.[31-33] Release of prostacyclin to compensate for vasoconstriction has been shown with sympathoadrenergic and neurohormonal activation. In humans vasoconstriction mediated by prostaglandin $F_{1\alpha}$ receptor releases urinary prostaglandins in response to infusion with norepinephrine,[34] and in an animal model stimulation of the sympathetic nerves leads to a significant rapid increase in output of prostacyclin from the mesenteric arterial bed.[35] The importance of vasodilatory prostaglandins in maintaining circulatory homoeostasis is evident in congestive heart failure, in which plasma concentrations of prostaglandin I_2 and prostaglandin E_2 metabolites correlate with the degree of activation of the neurohumoral vasoconstrictor systems.[36]

Although cigarette smoking is closely associated with peripheral arterial disease and atherosclerosis occurs first in the abdominal and iliac aortas, we assessed the grade of atherosclerosis in the carotid arteries owing to easier access in the neck and the limitations of ultrasonography in accurately detecting the state of arteries in the legs. Prostacyclin biosynthesis is appreciably increased in patients with severe atherosclerosis and activation of platelets.[18] In this study the smokers had more pronounced atherosclerotic changes than the non-smokers, but the changes were not severe enough to cause clinical manifestations and were not associated with the excretion of prostacyclin and thromboxane metabolites. Thus the finding of high urinary concentrations of the prostaglandin and thromboxane B_2 metabolites suggests that cigarette smoking can cause release of excessive vasoactive prostanoids to the circulation.

In conclusion, the study shows that chronic cigarette smoking can enhance the progression of atherosclerotic lesions in carotid bifurcations even without hyperlipidaemia. The increased excretion of the main metabolites of vasoactive prostanoids among smokers points to a dysfunction of both vascular walls and platelets. We have thus confirmed earlier findings[27] by studying pairs of subjects who are genetically identical. The urinary concentrations of the thromboxane B_2 metabolite were closely associated with the intensity of smoking, thus implying direct effects of smoking tobacco on activation of platelets. The increased biosynthesis of thromboxane A_2 additionally enhances the other vasoconstrictive properties of smoking. As prostacyclin is known to be a reactive vasodilator in regulating the circulation under various circumstances we propose that it compensates for the profound vasoconstrictive effects of cigarette smoking.

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