PHARMACODYNAMICS AND DRUG ACTION

Transdermal nicotine mimics the smoking-induced endothelial dysfunction

Background: Cigarette smoking is a major risk factor for coronary artery disease and causes endothelial dysfunction, perhaps by decreasing the availability of nitric oxide availability in arteries and veins. Nicotine in cigarette smoke may be responsible for this impaired endothelial response.
Methods: We studied nine healthy nonsmokers and 12 healthy mild to moderate smokers by use of the dorsal hand vein compliance technique. Dose-response curves to bradykinin and sodium nitroprusside were obtained to test the endothelium-dependent and endothelium-independent vasorelaxation before and during the use of a nicotine (21 mg) patch. Mean arterial blood pressure and heart rate were measured beat-to-beat during the 4-hour study and serial blood samples were drawn to assay plasma thromboxane B_2 levels.
Results: Transdermal nicotine reduced the venous responsiveness to bradykinin in nonsmokers (E_{max} = 88.0% ± 17.9% and 54.3% ± 14.9%, respectively, before and after the nicotine patch; $P < .05$); the latter response was similar to that in smokers (E_{max} = 56.3% ± 16.6%). Sodium nitroprusside–induced venodilation was unaltered. Mean arterial blood pressure increased in both smokers and nonsmokers. Transdermal nicotine increased the plasma thromboxane B_2 concentrations only among nonsmokers.
Conclusion: These findings indicate that nicotine can have a major role in the impaired endothelial function in smokers. The results probably also reflect what occurs in arterial beds because the nicotine patches increased the mean arterial blood pressure in both smokers and nonsmokers. (Clin Pharmacol Ther 2000;68:167-74.)

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Cigarette smoking, a major risk factor for coronary artery disease,[1] is associated with endothelial dysfunction.[2] In addition to the nicotine-dependent stimulation of the sympathetic system and the inactivation of vagal cardiovascular control,[3,4] smoking also decreases the availability of endothelium-derived nitric oxide in arteries[5-10] and veins.[11,12] Indeed, heavy smokers have an impaired venodilation to bradykinin, and smoking cessation results in restoration of the normal response in less than 48 hours.[2] Although the precise mechanism of smoking-induced endothelial dysfunction is unknown, nicotine is among the numerous compounds contained in cigarette smoke that could contribute to this impaired response in heavy smokers. However, studies have demonstrated that transdermal administration of nicotine in doses greater than 21 mg is safe for such patients.[13]

Because the number of mild and moderate smokers who are trying to stop smoking by using transdermal

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Table I. Clinical characteristics of the study subjects

	Nonsmokers	*Smokers*
Sex		
Men	6	6
Women	3	6
Age (y)	35.4 ± 4.2	38.3 ± 7.1
Weight (kg)	77.5 ± 9.6	71.8 ± 8.8
Years of tobacco use	—	12.4 ± 5.2
No. of cigarettes/day	—	12.2 ± 5.1
Fagerström score	—	3.5 ± 1.2
Expired carbon monoxide (ppm)	3 ± 2	9 ± 4*

Data are mean values ± SEM.
*$P < .05$ versus nonsmokers.

nicotine is increasing,[14] we investigated the role of nicotine in the impaired vascular responsiveness in this group of smokers. Because nicotine causes sympathetic activation,[4] we also evaluated the effects of this drug on blood pressure and heart rate. Finally, we measured the plasma concentrations of thromboxane B_2 (the stable breakdown product of thromboxane A_2) to assess whether transdermal nicotine affects the production in vivo of thromboxane A_2, a potent vasoconstrictor with platelet-aggregating activity that is involved in a number of cardiovascular conditions.[15]

METHODS

Study subjects. This study was approved by the Ethics Committee of the Faculty of Medical Sciences of the State University of Campinas, and each subject provided informed written consent. The subjects, who were nonsmokers (n = 9) or mild to moderate smokers (n = 12), provided a complete health history and underwent a physical examination, electrocardiography, and laboratory analysis to exclude individuals with dyslipidemia, diabetes mellitus, and evidence of hepatic, renal, or hematologic dysfunction. The Fagerström score[16] was determined to measure the degree of physical dependence on tobacco and correlates with other proposed measures of nicotine dependence (carbon monoxide, nicotine, and cotinine levels) (Table I).

Study protocol. The study was a placebo-controlled and crossover design in which the subjects were unaware of whether they received placebo or nicotine. The subjects were admitted to the university hospital on two different occasions for a 4-hour study. Smokers were encouraged to smoke cigarettes 4 hours before the study to make evident the possible effects of nicotine on the bradykinin receptors. Expired carbon monoxide was measured with a carbon monoxide monitor immediately before any procedures. An indwelling venous catheter was inserted for blood sample collection, and the subjects remained in the supine position thereafter. Subjects were randomized to treatment with either transdermal nicotine 21 mg (Nicoderm; Alza, Palo Alto, Calif) or placebo after they had been in the supine position for at least 30 minutes.

Dorsal hand vein technique. The dorsal hand vein reactivity method has been described in detail previously.[17,18] In brief, subjects remained in the supine position, and the room temperature was controlled (21°C to 23°C). A 23-gauge butterfly needle was inserted into a suitable dorsal hand vein, and a continuous infusion of physiological saline solution (0.317 mL/min) was started. The arm was placed on a support sloping upward at an angle of 30 to 45 degrees from horizontal to ensure complete emptying of the superficial hand veins. After 30 minutes a linear variable differential transducer (LVDT model 100 MHR; Schaevitz Engineering, Pennsauken, NJ) was mounted on the back of the hand with a tripod. When the freely movable core of the linear variable differential transducer was properly centered within the transformer and placed on top of the vein, there was a linear relationship between the vertical movement of the core and the voltage output over the range used. All responses were recorded on a strip chart recorder. Recordings were made before and after the inflation of a sphygmomanometer cuff to 40 mm Hg on the same arm. Results were expressed as normalized dose-response curves in which the diameter of the vein during saline solution infusion with the cuff inflated corresponded to 100% relaxation. To study the effects of vasodilator substances, the vein was preconstricted by infusion of increasing doses of the α_1-adrenergic selective agonist phenylephrine (99 to 3166 ng/min) into the 23-gauge needle butterfly until the dose that produced approximately 75% to 80% constriction of the vein (ED_{80}) was found. This dose of phenylephrine was then maintained throughout the study of venorelaxant drugs, including during the washout period between two dose-response curves. This degree of preconstriction was defined as 0% dilation. The venodilation produced by bradykinin (1 to 278 ng/min) and sodium nitroprusside (0.01 to 634 ng/min) during use of the transdermal nicotine or placebo was calculated as a percentage of the range between maximal (100%) and 0% vasodilation. A dose-response curve to bradykinin was constructed with five infusion rates ranging from 1 to 278 ng/min^2. Infusions at each rate lasted for 5 minutes with the sphygmomanometer cuff inflated to 45 mm Hg for the last 2 minutes of the infusion.

Noninvasive blood pressure monitoring. Beat-to-beat noninvasive mean finger arterial blood pressure (MABP) and heart rate were monitored with a Finapres

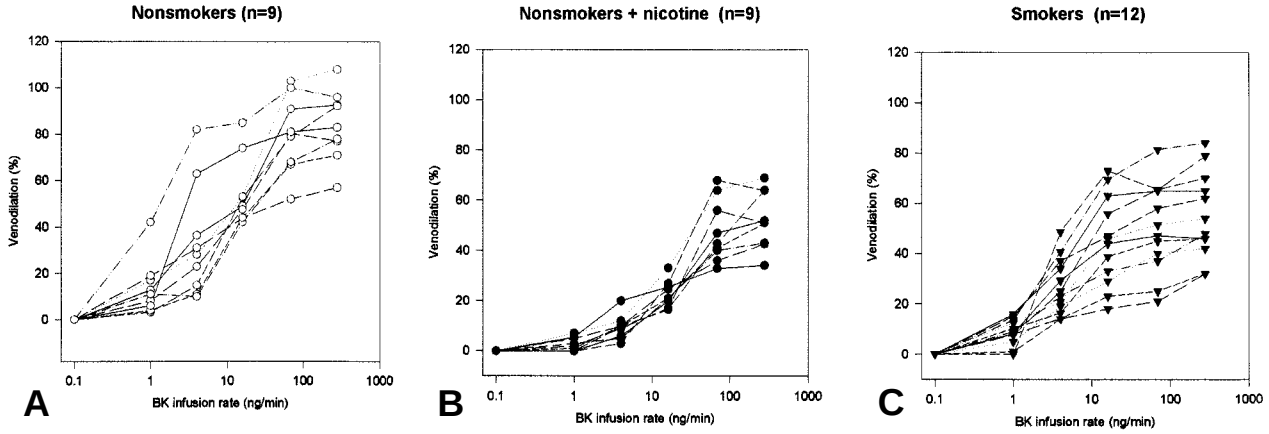

Fig 1. Dorsal hand venodilation (%) in response to bradykinin (BK; 0.1 to 278 ng/min) in nonsmokers with placebo patch **(A)** or nicotine patch **(B)** and in mild to moderate smokers **(C)**. The dorsal hand veins were preconstricted with phenylephrine, and the venodilation was expressed as a percentage of the baseline (prephenylephrine) vein diameter.

2300 system (Ohmeda, Englewood, Colo).[19] In brief, this device, which is based on the arterial volume clamp method, measures blood pressure through a small finger cuff wrapped around the middle finger of either hand. The MABP and heart rate values were recorded every 10 seconds throughout the 4-hour study period. The average values recorded during 5 minutes around predetermined time points were used to compare MABP and heart rate results. Simultaneously, an ambulatory blood pressure system (SpaceLabs, Redmond, Wash) was used in the opposite arm to check the Finapres measurements every 5 minutes.

Thromboxane B_2 assays. Venous blood samples were collected in tubes containing ethylenediamine tetraacetic acid at baseline and at 30 minutes, 1 hour, and 4 hours after application of the nicotine or placebo patch. The plasma was separated by centrifugation and stored at –20°C until assayed. Plasma samples were extracted by use of C_{18} reversed-phase cartridges (Waters Co, Milford, Mass), and the thromboxane B_2 levels were determined by use of a commercial enzyme immunoassay (Cayman Chemical Co, Ann Arbor, Mich).

Statistical analysis. The results were expressed as means ± SEM. The bradykinin dose-response curves were fitted to a sigmoid model,[20] and the maximum effect (E_{max}) and the bradykinin dose, which cause 50% of the E_{max} (ED_{50}), were determined. Parametric tests (Student paired and unpaired *t* tests) were used to compare the E_{max} and $logED_{50}$ values. Other parameters (heart rate, MABP, and thromboxane B_2) were analyzed by ANOVA for repeated measurements. $P < .05$ was considered the minimum level for statistical significance.

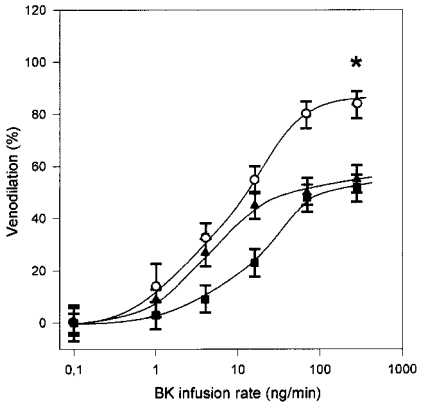

Fig 2. Dose-response curves to bradykinin. *Open circles,* Nonsmokers after placebo patch (n = 9); *solid circles,* nonsmokers after nicotine patch (n = 9); *solid triangles,* mild to moderate smokers (n = 12). Curves for placebo (n = 3) or nicotine (n = 4) patches in smokers (not shown) were almost superposed on those of smokers with no patches *(solid triangles).* The points are the mean ± SEM of the data in Fig 1. The curves were fitted as described in Parati et al.[19] *$P < .05$ versus nonsmokers with placebo patch (E_{max}).

Drugs. Phenylephrine hydrochloride (1% solution) was from Winthrop Laboratories (New York, NY), bradykinin was from Clinalfa (Laeufelfingen, Switzerland), sodium nitroprusside was from Elkins-Sinn Inc (Cherry Hill, NJ), and transdermal nicotine (21 mg; Nicoderm) was from Alza (Palo Alto, Calif). All drugs were diluted in normal sterile saline solution before use.

Table II. Maximum response to bradykinin (percent of maximum) and $logED_{50}$ obtained from complete dose-response curves (0.1 to 278 ng/min) in nonsmokers and mild to moderate smokers

	Nonsmokers		*Mild to moderate smokers*	
	Placebo (n = 9)	*Nicotine (n = 9)*	*Placebo (n = 12)*	*Nicotine (n = 12)*
E_{max} (%)	88.1 ± 17.9	54.3 ± 14.9*	56.0 ± 16.6†	48.3 ± 13.7†
$LogED_{50}$	1.1 ± 0.1	1.2 ± 0.6	0.7 ± 0.2	0.8 ± 0.4

Data are mean values ± SEM.
*$P < .05$ versus placebo patch.
†$P < .05$ versus placebo patch in nonsmokers.

Table III. Maximum response to sodium nitroprusside (percent of maximum) and $logED_{50}$ obtained from complete dose-response curves (0.01 to 634 ng/min) in nonsmokers and mild to moderate smokers

	Nonsmokers		*Mild to moderate smokers*	
	Placebo (n = 9)	*Nicotine (n = 9)*	*Placebo (n = 12)*	*Nicotine (n = 12)*
E_{max} (%)	107 ± 23	96 ± 32	96 ± 25	106 ± 42
$LogED_{50}$	1.3 ± 0.2	1.4 ± 0.6	0.9 ± 04	1.6 ± 0.7

Data are mean values ± SEM.

RESULTS

Characteristics of the study groups. Table I summarizes the basic characteristics of the subjects studied. There were no significant differences in the age and weight of smokers and nonsmokers. The smokers had an average Fagerström score[16] of 3.5, indicating the degree of addiction to cigarette smoking. In addition, smokers presented higher levels of expired carbon monoxide as compared with nonsmokers.

Effects of nicotine on venous endothelial function. None of the drug infusions resulted in significant changes in blood pressure or heart rate, indicating a lack of systemic effect of the doses used. Transdermal nicotine decreased the dorsal vein responsiveness to bradykinin in nonsmokers (E_{max}, 88.0% ± 17.9% and 54.3% ± 14.9%, after placebo and transdermal nicotine, respectively; $P < .05$; Fig 1, *A* and *B*, Fig 2, and Table II). This impairment was similar to that observed in smokers compared with nonsmokers with transdermal nicotine (E_{max}, 48.4% ± 13.7% and 54.3% ± 14.9%, respectively; $P > .05$; Fig 1, *B* and *C*, Fig 2, and Table II). Transdermal nicotine did not attenuate sodium nitroprusside–induced venodilation in smokers (E_{max}, 107% ± 23% and 96% ± 32% for placebo and transdermal nicotine, respectively). Similarly, the ED_{50} values for sodium nitroprusside were not different between smokers and nonsmokers using placebo or transdermal nicotine (Table III).

Changes in MABP and heart rate. The results for MABP are shown in Fig 3, *A* and *B*, and those for heart rate are shown in Fig 3, *C* and *D*. The placebo did not change the MABP or heart rate in either group, whereas transdermal nicotine increased MABP after 1 hour (from 87 ± 4 to 111 ± 5 mm Hg) in nonsmokers and after 2 hours (from 83 ± 2 to 98 ± 6) in smokers ($P < .05$, both). The heart rate increased after 1 hour (from 69 ± 2 to 82 ± 3 beats/min, $P < .05$) of transdermal nicotine use only in nonsmokers. This increase in MABP and heart rate was accompanied by nausea, lightheadedness, mild headache, and sweating in nonsmokers, thus precluding the continuation of the experiments for much more than 1 hour in these subjects.

Plasma thromboxane B_2 levels. There were no differences between the basal thromboxane B_2 plasma levels in nonsmokers and smokers (554 ± 102 and 643 ± 186 pg · mL^{-1}, respectively; P = NS). The use of transdermal nicotine for 1 hour increased thromboxane B_2 concentrations only among nonsmokers (up to 1166 ± 243; $P < .05$); there were no changes among the smokers throughout the 4-hour study period (Fig 4).

DISCUSSION

The main finding of this investigation was that transdermal nicotine administration to nonsmokers blunted the vasodilator response to bradykinin compared with that in smokers. This observation strongly suggests a pivotal role for nicotine in endothelial dysfunction in cigarette smokers. The blunted response to bradykinin and an unimpaired response to sodium nitroprusside after nicotine administration are indicative of endothe-

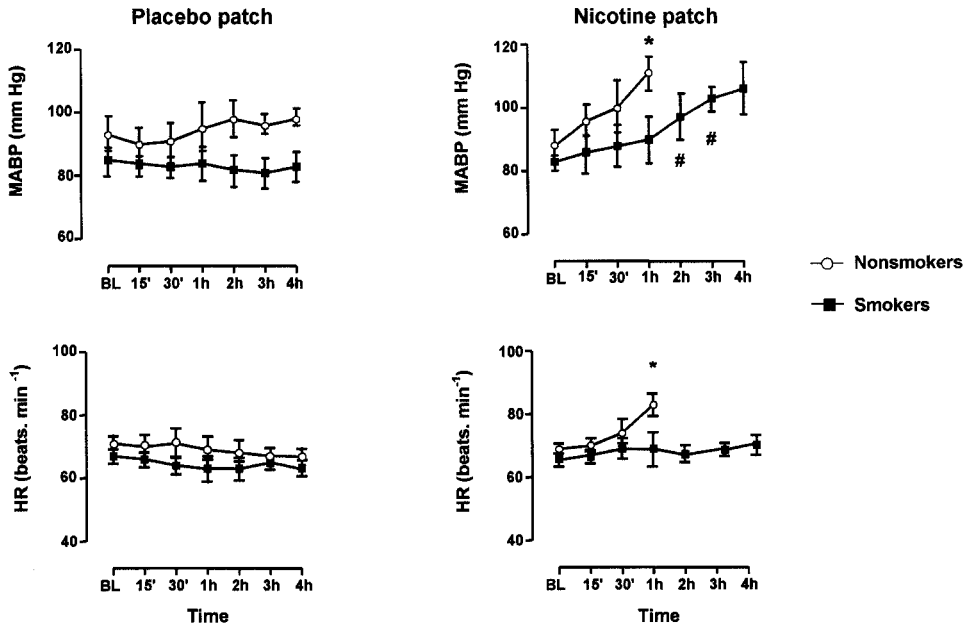

Fig 3. Mean finger arterial blood pressure (MABP) and heart rate (HR) before (BL) and up to 4 hours after placebo and nicotine patches in nonsmokers *(open circles;* n = 9) and mild to moderate smokers *(solid squares;* n = 12). The points are the mean ± SEM. **P* < .05 versus BL in the nonsmoker group. #*P* < .05 versus BL in the group of mild to moderate smokers.

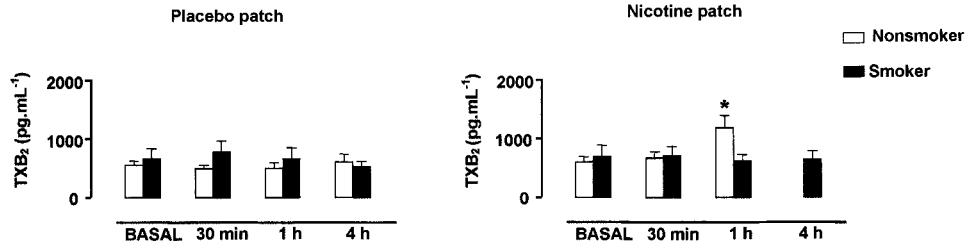

Fig 4. Plasma thromboxane B_2 (TxB_2) concentrations before (BASAL) and after placebo or nicotine patch application in nonsmokers *(open bars;* n = 9) and mild to moderate smokers *(solid bars;* n = 12). The *bars* are the mean ± SEM. *P* < .05 versus BASAL in the nonsmoker group.

lial dysfunction and not of a hyporeactivity of dorsal hand vein vascular smooth muscle to nitric oxide. A direct and local effect of nicotine has been recently demonstrated by Chalon et al.[21] Now, for the first time, our findings showed arterial systemic effects caused by transdermal nicotine. The design of this study did not permit us to assess whether prostacyclin or nitric oxide production was decreased in the subjects who used transdermal nicotine.

Habitual cigarette smoking is associated with signs of sympathetic predominance in the autonomic control of the sinoatrial node.[22] However, nicotine produces only minor disturbances of autonomic regulation in smokers.[22] Thus the unaltered heart rate seen here in smokers and described by others,[22] together with the clear endothelial dysfunction in veins, suggest a direct, systemic effect of nicotine on arterial resistance, as demonstrated by the increase in MABP in nonsmokers and smokers.

The presence of acetylcholine receptors sensitive to nicotine has been reported in cultured endothelial cells from human aorta.[23] These receptors have ion-gating properties similar to those found in neuronal ganglionic acetylcholine receptors. Thus the stimulation by nicotine of acetylcholine receptors in endothelial cells may mediate the effects of nicotine in the vascular system.[23]

In this study such effects occurred earlier in nonsmokers than in mild to moderate smokers, probably because the long-term use of nicotine favors the development of tolerance to the acute effects of this drug. In support of this hypothesis, increasing doses of nicotine (21, 42, and 63 mg/day) did not change the heart rate and MABP in heavy smokers, a finding consistent with the development of tolerance.[13]

A recent study has suggested that nicotine alters the dilation of arterioles via an increased release of superoxide anion and subsequent inactivation of nitric oxide.[24] In addition, studies in arteries in vitro have shown that free radicals in cigarette smoke extract increase the degradation of nitric oxide.[25,26] Indeed, free radical-induced oxidative damage is believed to be involved in the pathogenesis of arterial diseases associated with cigarette smoking.[27] Although nicotine impairs endothelium-dependent vasodilation, the unaltered responses to sodium nitroprusside we found after nicotine infusion strongly suggest that endothelium-independent mechanisms were not affected.

Early[28,29] and more recent[30] reports provide evidence that long-term tobacco use causes depressed baroreceptor sensitivity in smokers. Furthermore, an overnight cessation of smoking is associated with an increase in the sympathetic activity of the vascular system in the morning, which is suppressed by smoking the first cigarette.[31] This effect of smoking decreases in the afternoon after continuous nicotine use. Such a phenomenon could explain the late increase in MABP seen in the mild to moderate smokers here compared with heavy smokers.[13]

Increased levels of thromboxane B_2 in nonsmokers after use of transdermal nicotine may reflect an increased platelet activation.[32] This finding agrees with a previous study demonstrating increased excretion of thromboxane B_2 in smokers.[32] On the other hand, an intravenous infusion of nicotine for 30 minutes in nonsmokers was not accompanied by a significant release of thromboxane A_2, suggesting that nicotine produces weak platelet stimulation.[33] In agreement with this finding, we observed stable thromboxane B_2 levels in nonsmokers for 30 minutes after the administration of transdermal nicotine. However, the thromboxane B_2 levels increased significantly after 1 hour of transdermal nicotine use. This response may reflect the gradual increase in serum nicotine levels when transdermal nicotine is used.[34]

Some methodologic aspects of this study must be taken into account. First, although veins do not respond in the same way as arteries, our findings on vascular function may be extended to arteries because venous and arterial endothelium have the same embryologic origin.[35] Previous studies with techniques such as reactive hyperemia[5,36-38] and plethysmography[39-41] have reported conclusions comparable to those reached with the dorsal hand vein technique.[2,42] This safe[17] technique allows investigations of vessels without the gross morphologic alterations found in arterial atherosclerosis frequently observed in smokers.[43,44] Second, the lack of continuous (24-hour) arterial blood pressure and heart rate data, especially when the rate of nicotine absorption is maximal (normally 6 to 12 hours after the patch is applied[34]), limits the interpretation of the late effects of nicotine in mild and moderate smokers. In addition, the quantification of 24-hour urinary catecholamine levels would have been useful for evaluating the role of the adrenergic system in our subjects.

In conclusion, our results demonstrate that nicotine has a pivotal role in the impaired endothelial function seen in the dorsal hand veins of smokers. This interference may occur via a direct, systemic effect of this drug on vascular systemic resistance as shown by the increase in the MABP in nonsmokers and smokers. The relative importance of the direct effects of nicotine on endothelial cells and in autonomic regulation deserves further studies, as does the possible modulation of endothelium-derived mediators by nicotinic receptors.

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