Effects of transdermal nicotine treatment on structure and function of coronary artery bypass grafts

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Clouse, William D., Hiroki Yamaguchi, Michael R. Phillips, Richard D. Hurt, Lorraine A. Fitzpatrick, Thomas P. Moyer, Charles Rowland, Hartzell V. Schaff, and Virginia M. Miller. Effects of transdermal nicotine treatment on structure and function of coronary artery bypass grafts. J Appl Physiol 89: 1213–1223, 2000.—Smoking is a major risk factor for failure of coronary artery bypass grafts (CABG). Experiments were designed to determine effects of transdermal nicotine, independent of smoking, on structure and function of CABG. Saphenous veins were placed as CABG in untreated dogs (control) or in dogs treated with transdermal nicotine (one 11-mg or two 22-mg patches/day) for 5 wk. Serum nicotine and plasma nitric oxide were measured. Grafts were removed and prepared for organ chamber studies and histology. Serum nicotine averaged 12.1 and 118.7 ng/ml in the 11 mg/day and 44 mg/day groups, respectively. Plasma nitric oxide was higher in dogs treated with 11 mg/day doses compared with controls. In organ chamber studies, endothelium-dependent relaxations to thrombin and A-23187 and endothelium-independent relaxations to nitric oxide were greatest in grafts from dogs treated with 11 mg/day doses. Intimal thickness of the grafts were similar among groups. However, staining for bone sialoprotein was increased in the media of grafts from dogs treated with 11 mg/day treatment group. These data suggest that transdermal nicotine treatment increases nitric oxide production of and response to nitric oxide in bypass grafts.

CIGARETTE SMOKING IS A RISK factor for failure of coronary artery bypass grafts (CABG) and decreases survival in patients with previous CABG (9, 15, 45). Nicotine is the addictive component of cigarette smoke, and nicotine replacement products, such as patches, gum, inhaler, and nasal spray, are being used to aid smokers in stopping smoking. Two of these products (nicotine patches and gum) are approved by the Food and Drug Administration for over-the-counter use. Despite the known beneficial effects of smoking cessation to reduce the risk of significant coronary events, there is a reluctance on the part of many physicians to prescribe nicotine treatment for smoking cessation in surgical patients because the cardiovascular consequences of nicotine treatment independent of smoking remain unknown. Two prospective clinical studies suggest that nicotine treatment for smoking cessation does not increase the number of primary coronary events in high-risk populations (21, 46). However, there are no controlled pharmacological studies that have examined specifically the effects of transdermal nicotine on function of CABG in the absence of cardiovascular risk factors or smoking. Nicotine is being used clinically to treat non-smoking-related disorders such as ulcerative colitis, Parkinson’s disease, Alzheimer’s disease, and sleep disorders (12, 14, 37, 39, 41). Therefore, understanding how nicotine affects the structure and function of bypass grafts in the absence of smoking may provide insight into how nicotine might ameliorate symptoms in other conditions. Experiments were designed to characterize effects of transdermal nicotine therapy on function and morphology of reversed saphenous vein CABG in dogs in the subacute postoperative period (4 wk). On the basis of results of clinical trials (21, 46), it was hypothesized that treatment with transdermal nicotine would not decrease patency rates of CABG in dogs in the subacute postoperative period. Furthermore, on the basis of results of effects of nicotine on endothelial function in experimental animals and cultured endothelial cells (3, 6, 28, 35, 42, 43), it was hypothesized that changes in endothelial function would be dependent on the dose of nicotine treatment. Transdermal nicotine was tested at doses within and double to those used for conventional smoking cessation programs (7–22 mg/day) in humans (11).

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METHODS

Animals. Sixteen male mongrel dogs (19–27 kg) were used in this study: six were not treated with nicotine patches (control), five were treated with 11 mg/day nicotine patches (Prostep, Elan Pharmaceuticals, Athlone, Ireland), and five were treated with two 22 mg/day patches. Dogs’ necks were shaved, and patches were applied to the skin. Loose-fitting leather collars were used to prevent patch removal by scratching. Doses in the 44 mg/day group were sequentially increased by starting at 11 mg/day on the first day of therapy and reaching 44 mg/day on the sixth day of treatment.

One week after the initiation of nicotine patch therapy, reversed saphenous veins were placed as CABG from the left subclavian artery to the circumflex coronary artery without cardiopulmonary bypass, as previously described (32). The bypassed segment of coronary artery was ligated so that blood flow to the distal coronary artery was graft dependent. Anesthesia was induced with methohexital sodium (12–15 mg/kg iv) with isoflurane (1–3%) inhalation anesthesia (positive pressure, tidal volumes of 10–15 ml/kg) with supplemental midazolam (5–10 mg/dose) and fentanyl (50–100 µg/dose). Hydration was maintained with PlasmaLyte-A balanced salt solution (10–15 ml·kg⁻¹·h⁻¹). One unit of homologous whole blood (300–400 ml) was given during placement of the graft. A femoral arterial catheter was placed percutaneously for monitoring of blood pressure. Preoperative antibiotics for prophylaxis consisted of gentamicin (80 mg iv), cefazolin (1 g iv), and penicillin G procaine (1.2 million units/ml). Enteric-coated aspirin (325 mg/day) was administered to all dogs starting 2 days preoperatively and continued for the duration of the study, as is standard of care for humans undergoing bypass surgery.

Torbugesic (10 mg/dose) was used for postoperative analgesia, and acepromazine (2 mg/dose) was used for postoperative sedation. Dogs received oral cefadroxil (25 mg/kg bid) for 3 days postoperatively. All animal care was in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health (NIH Publication No. 86–23, revised 1985).

In vitro studies. Four weeks postoperatively (5 wk of nicotine patch therapy), animals were anesthetized (pentobarbital sodium 30 mg/kg iv) and exsanguinated via the carotid arteries. Grafts were exposed through a median sternotomy. The heart and great vessels were harvested en bloc with the graft intact. Graft patency was confirmed by transecting the distal anastomosis and by the absence of infarcted lateral myocardium in cross section. Grafts were dissected from connective tissue and quickly placed in chilled modified Krebs-Ringer bicarbonate solution of the following composition (mmol/l): 118.3 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.22 KH₂PO₄, 2.5 CaCl₂, 25 NaHCO₃, and 11.1 glucose (control solution).

Organ chambers. Rings (5 mm in length) were cut from the middle portion of the grafts. The endothelium was removed intentionally from some rings by gently rubbing the internal surface with watchman’s forceps.

Rings were suspended in organ chambers (25 ml) filled with control solution at 37°C and gassed with 95% O₂:5% CO₂. Two stainless steel wire stirrups were placed through the lumen of the graft segments. One stirrup was anchored to the bottom of the chamber and the other to a force transducer (Gould UC2, Cleveland, OH) for measurement of isometric force. Rings were placed at the optimal point on the length-tension curve by stepwise stretching and contracting with 20 mM KCl at each level of stretch. Once at optimal length (baseline tension), rings were equilibrated for 30 min, and contractions to 60 mM KCl were measured.

Cumulative concentration-response curves were obtained to the α₁-adrenergic agonist UK-14304 (10⁻⁵–10⁻⁶ mol/l) from baseline tension. To study relaxations, rings were first contracted with PGF₂α (2 × 10⁻⁶ mol/l). When contractions plateaued, cumulative concentration-response curves were obtained to either ADP (10⁻⁶–10⁻⁴ mol/l), thrombin (10⁻²–1.0 units/ml), or the calcium ionophore A-23187 (10⁻⁶–10⁻⁴ mol/l) in rings with endothelium and to nitric oxide (3 × 10⁻⁶–10⁻⁵ mol/l) in rings without endothelium. UK-14304 was studied to assess changes in adrenergic receptor sensitivity associated with denervation of the saphenous veins (16). ADP and thrombin were studied because endothelium-dependent relaxation to these agonists is preserved in vein grafts in dogs and humans (27, 36) and are associated with release of nitric oxide. Calcium ionophore releases endothelium-derived factors independent of receptor activation and, therefore, provides an assessment of calcium-dependent endothelial function. All experiments were performed in the presence of the cyclooxygenase inhibitor indomethacin (10⁻⁵ mol/l) because animals were treated with aspirin throughout the experimental period. Therefore, changes in endothelium-dependent responses in vitro were associated with factors other than prostanooids, including prostacyclin and thromboxane. Rings with and without endothelium were studied in parallel in the absence and presence of the arginine analog N⁶-monomethyl-L-arginine (L-NMMA, 10⁻⁴ mol/l) to inhibit production of nitric oxide. Grafts were incubated with L-NMMA for 30 min before the initiation of experiments. Rings were washed with control solution at least three times between dose-response curves and equilibrated for at least 30 min before initiation of the next curve. Once L-NMMA was added to the bath, it was readded before each dose-response curve. Exogenous nicotine was not added to organ baths in any experiments.

Drugs. The following drugs were used during the experiments: adenosine diphosphate, calcium ionophore A-23187, dimethyl sulfoxide, L-NMMA, nicotine, PGF₂α, indomethacin (Sigma Chemical, St. Louis, MO), and UK-14304 (Pfizer Central Research, Sandwich, Kent, UK). Drugs were prepared daily in distilled water and were kept refrigerated. The calcium ionophore was dissolved in dimethyl sulfoxide (final bath concentration of 8.2 × 10⁻³ mol/l) and diluted in distilled water. Indomethacin was prepared with an equal molar amount of Na₂CO₃ in distilled water (10⁻⁵ mol/l). Dimethyl sulfoxide and Na₂CO₃ have no effects on response in vein bypass grafts (7, 25). All concentrations refer to final molar concentrations (mol/l) of the drug in the organ chamber.

Blood samples. Venous blood samples were taken before initiation of nicotine patch therapy (baseline), at 6 and 24 h after patch application 1 day preoperatively, and on postoperative days 1, 7, 14, 21, and 28. Six hours after patch application was chosen for sampling because pharmacokinetic studies in humans showed that peak nicotine levels with this delivery system occur at about this time point. All blood samples were centrifuged (3,200 g for 15 min at 5°C) immediately. Plasma for the determination of nitric oxide was drawn into a syringe and transferred into a siliconized vacuum tube for storage at −70°C. Cotingine, the major metabolite of nicotine, was measured in plasma by HPLC (29). Nicotine was measured in serum by gas chromatography-mass spectrometry (2). Oxidized products of nitric oxide (NOx) were measured by chemiluminescence (Sievers nitric oxide analyzer model 270B, Boulder, CO) (4).

Histological analysis. Rings from proximal, middle, and distal sections of grafts, adjacent to those used in organ
chamber studies, were fixed in 10% formalin solution and prepared for light microscopy. Cross sections of grafts (6 μm) were stained with elastin van Gieson’s stain.

Adjacent sections of grafts were stained immunohistochemically for smooth muscle α-actin (murine monoclonal IgG1 anti-human muscle actin, HHF35, DAKO, Carpinteria, CA), desmin (murine monoclonal IgG anti-swine desmin, DE-R-11, DAKO), Ki-67 nuclear antigen (murine monoclonal IgG1 MIB-1 antibody against Ki-67 nuclear antigen found only in cells outside G0 phase; Immunotech, Westbrook, ME) and bone sialoprotein (BSP, polyclonal rabbit, LF-100; gift from Dr. Larry Fisher, National Institutes of Dental Research, National Institutes of Health, Bethesda, MD). Actin and desmin were used to identify differentiated contractile smooth muscle cells, Ki-67 identifies dividing cells, and bone sialoprotein is a noncollagenous matrix protein that may be related to vascular calcification. Vectastatin ELITE ABC kit (Vector Laboratories, Burlingame, CA) was used for sections stained for BSP. Hematoxylin was used as counterstain for all other antibodies. To control for nonspecific staining, adjacent sections were stained with IgG in the absence of the primary antibody. Immunostaining was performed in the Immunohistochemical Laboratory and Bone Morphometric Laboratory at the Mayo Clinic. In general, paraffin was removed from the tissue sections by using xylene (100%). Tissue was rehydrated into descending ethanol and blocked in Tris-buffered saline (0.05 M Tris, 0.01% BSA, 0.9% NaCl, pH 7.5) containing 0.3% casein and 10% normal goat serum. Incubations with primary and secondary antibody (biotinylated goat anti-rabbit) were performed at room temperature followed by two 15-min washes in Tris-buffered saline containing 0.02% Triton X-100. Endogenous peroxidase activity was inhibited with 1.5% H2O2 in 0.1% sodium azide and 50% methanol for 15 min. Bound secondary antibody was detected with peroxidase-conjugated avidin-biotin complex and visualized with the use of 0.05% diaminobenzidine and 0.01% H2O2. Sections were rinsed with tap water, dehydrated with ascending alcohols, and cleared with xylene.

Statistical analysis. Data are expressed as means ± SE. The n represents the number of grafts from different dogs. Contractions to UK-14304 are expressed as a percentage of contractions to 60 mM KCl. Relaxations are expressed as a percentage of the contraction to PGF2α. The concentration of drug causing 50% of maximal relaxation or contraction (ED50) was calculated for individual dose-response curves, and the mean was reported as the logarithm of the molar concentration. Areas under individual dose-response curves were calculated and averaged as the mean for all responses in a treatment condition. Maximal response, ED50, and area under the curve were compared within groups by Student’s t-test for paired observation and among groups by one-way ANOVA. Values were considered statistically significant if P ≤ 0.05.

For assessment of neointimal hyperplasia, measures of internal and external elastic lamina (mm) around the circumference of the graft and the amount of media and neointima on a section (medial and neointimal areas, respectively, mm2) were quantified by using an Axioshot Photomicroscope (Carl Zeiss, Oberkochen, Germany) equipped with a ×50/0.15-numerical aperture Plan-NEOFOL refractive lens. Brightfield images were digitized on an IBAS Image Analysis System (Kontron Elektronik, Munich, Germany) using a black and white Newvicon video camera (Hamamatsu, Tokyo, Japan). Images were captured at a resolution of 512 × 768 picture elements (pixels). Each image was analyzed by use of a macro computer program written with software supplied by the IBAS system.

Average intimal thickness was calculated as the quotient of the neointimal area divided by the length of the internal elastic lamina. Medial thickness was calculated as the quotient of the difference between combined medial and intimal area minus the intimal area divided by the length of the external elastic lamina. Intimal-to-medial thickness ratios were assessed. Intimal-to-medial area ratios were calculated as the area of the neointima divided by the combined area of the neointima and the media minus the area of the neointima. Assessment of immunological staining was based on a qualitative scale of extent and intensity of staining in the neointima, media, and adventitia. This assessment was not quantified for statistical analysis.

In general, for statistical analysis, Student’s t-test was used to compare observations within a group. When more than two groups were compared, one-way ANOVA was used. If significant differences were found, Bonferroni’s post hoc test for multiple comparisons was used to identify these differences. Values were considered statistically significant if P ≤ 0.05.

RESULTS

Surgical outcome. Fifteen of 16 grafts were patent at 4 wk postoperatively. One graft occluded (dog receiving 44 mg/day dose) as a result of technical problems, which occurred while the proximal anastomosis was being performed. This graft was excluded from the study.

Blood analysis. Nicotine and its major metabolite in humans, cotinine, were not detectable in blood from control dogs (n = 6). Nicotine and cotinine showed dose-dependent increases in serum levels 6 h after application of the patches and decreased by 24 h after application of the patches. Peak steady-state values (6-h values averaged for weeks 2–5 of treatment) for plasma nicotine averaged 12.1 ± 6.7 ng/ml (n = 5) and 118.7 ± 38.5 ng/ml (n = 5) in the 11 mg/day and 44 mg/day groups, respectively. Peak steady-state values for plasma cotinine averaged 0.2 ± 2.2 (n = 5) and 35.1 ± 4.1 ng/ml (n = 5) in the 11 mg/day and 44 mg/day respectively over the four postoperative weeks. Plasma NOx before treatment with nicotine was similar among groups (control 10.7 ± 0.5 nmol/ml, n = 6; 11 mg/day group 11.8 ± 2.5 nmol/ml, n = 5; 44 mg/day group 9.3 ± 0.8 nmol/ml, n = 4). Six hours after patch application, NOx increased significantly 3 wk postoperatively in the 11 mg/day group and decreased toward baseline at the last week of study (Fig. 1). No statistically significant changes in plasma NOx were observed during weeks 1–5 in either the control or 44 mg/day groups (Fig. 1).

Organ chamber studies. Tension at optimal length (basal tone) did not differ significantly among groups and averaged 3.2 g (range 2.5–3.9) in rings with endothelium and 2.9 g (range 2.1–3.4) in rings without endothelium. L-NMMA caused contractions in three of six grafts with endothelium (0.7 ± 0.5 g; n = 3) from control dogs and in all grafts with endothelium from dogs treated with nicotine patches. These contractions were not significantly different among nicotine-treated dogs and averaged 1.3 ± 0.4 g in rings from both groups (n = 9).
Rings with and without endothelium from all grafts contracted to KCl and PGF\textsubscript{2\alpha}. Contractions of rings with endothelium to both KCl and PGF\textsubscript{2\alpha} were not different among groups (Table 1). Contractions of rings without endothelium to both agonists were significantly greater in grafts from dogs treated with the 44 mg/day dose compared with those from either untreated dogs or dogs treated with the 44 mg/day dose. This difference was eliminated by L-NMMA (−log ED\textsubscript{50} 7.5 ± 0.4 vs. 7.7 ± 0.3 in rings with and without endothelium, respectively; \(n = 4\)).

ADP caused comparable concentration-dependent relaxations in all rings with and without endothelium (Fig. 3). Relaxations to ADP were not statistically different among groups, nor were they altered significantly by incubation with L-NMMA (data not shown; \(n = 5–6\)).

Thrombin caused concentration-dependent relaxations in rings with endothelium from untreated dogs and from dogs treated with an 11 mg/day dose of nicotine (Fig. 4). Maximal relaxations were significantly greater in rings with endothelium from dogs treated with 11 mg/day dose compared with rings from untreated dogs. In contrast, relaxations to thrombin (1.0 units/ml) were significantly decreased by L-NMMA only in rings with endothelium from dogs treated with 11 mg/day dose (−25.6 ± 5.0% to −14.4 ± 5.2%; \(n = 5\)).

The calcium ionophore A-23187 produced concentration-dependent relaxations in rings with endothelium (Fig. 5). There were no statistically significant differences in relaxations between rings from control dogs and those from from dogs treated with the 44 mg/day dose. Relaxations with nitric oxide were not altered significantly by L-NMMA (data not shown, \(n = 4–6\)).

Histological analysis. No differences in neointimal thickness or ratios of intimal to medial area and intimal to medial thickness (not shown) were found among groups (Fig. 2). Contractions were significantly greater in rings without endothelium compared with those with (−log ED\textsubscript{50} 7.2 ± 0.3 and 7.6 ± 0.2, respectively; \(n = 4\); Student’s t-test for paired observation) only in rings from dogs treated with the 44 mg/day dose. This difference was eliminated by L-NMMA (−log ED\textsubscript{50} 7.5 ± 0.4 vs. 7.7 ± 0.3 in rings with and without endothelium, respectively; \(n = 4\)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>KCl (60 mmol/l)</th>
<th>PGF\textsubscript{2\alpha} (2 × 10\textsuperscript{-6} mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With endothelium</td>
<td>Without endothelium</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>((n = 6))</td>
<td>2.6 ± 0.3 g</td>
<td>1.6 ± 0.3 g</td>
</tr>
<tr>
<td>11 mg/day nicotine</td>
<td>3.0 ± 0.8 g</td>
<td>3.6 ± 0.9 g\textsuperscript{*}</td>
</tr>
<tr>
<td>((n = 5))</td>
<td></td>
<td></td>
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<tr>
<td>44 mg/day nicotine</td>
<td>2.5 ± 0.5 g</td>
<td>1.4 ± 0.4 g</td>
</tr>
<tr>
<td>((n = 4))</td>
<td></td>
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</tbody>
</table>

Values are as means ± SE; \(n\), no. of grafts from different dogs. 
\textsuperscript{*}Significantly different from control, \(P < 0.05\) (ANOVA).
grafs from untreated dogs compared with grafts from dogs treated with nicotine patches (Table 2).

Immunostaining for smooth muscle α-actin was present in neointimal myofibroblasts of all grafts. Stain for desmin was localized to the media and was not present in the neointima or in neointimal myofibroblasts. No differences in intensity of stain or distribution were observed among groups (Fig. 6). Positive staining for BSP was observed in sections from all grafts (Fig. 7). In grafts from control dogs and dogs treated with a 44 mg/day dose of nicotine, staining for BSP was more intense and was distributed in the media in addition to the neointima and adventitia (Fig. 7). Stain identifying proliferating cells (murine monoclonal antibody against Ki-67 nuclear antigen) was modest and found only in the adventitia of grafts, specifically around suture material (not shown).

**DISCUSSION**

Results of this study identify, for the first time, effects of transdermal nicotine independent of smoking on the function and structure of reverse saphenous vein CABG in an experimental animal. Doses and duration of application of the transdermal nicotine were comparable (11 mg/day) and double (44 mg/day) to those used for conventional smoking cessation programs in humans (11). In humans undergoing nicotine...
Patch therapy for smoking cessation, serum nicotine levels show substantial variability among subjects with levels ranging from 8 to 30 ng/ml with dosing up to 22 mg/day (1, 17, 19). Nicotine levels in smokers immediately after one cigarette per hour reach peak serum venous nicotine levels of 30 ng/ml (38). In this study, dogs treated with a 11 mg/day dose achieved levels similar to average smokers (~12 ng/ml), whereas dogs in the 44 mg/day group achieved concentrations much higher than those of heavy smokers (~118 ng/ml) (19). Cotinine, the major metabolite of nicotine via cytochrome P-450, showed 5- to 10-fold lower levels in the study dogs than in humans using nicotine patches (1, 11, 19). The reasons for this are unclear and may reflect atherosclerotic disease in smokers or other metabolic changes associated with smoking. Alternatively, there may be differences in P-450 enzymes of dogs compared with those of humans.

A positive finding of this study is that low but not high doses of nicotine therapy may stimulate changes in production of endothelium-derived relaxing factors in CABG, in particular nitric oxide. In support of this conclusion are observations that endothelium-dependent relaxations to thrombin and A-23187 were greater in grafts from dogs treated with an 11 mg/day dose of nicotine compared with those treated with a 44 mg/day dose. The fact that relaxations in grafts from the 11 mg/day treatment groups were decreased by l-NMMA suggests, albeit indirectly, that production of nitric oxide by endothelial cells may be increased by this dose of nicotine. The fact that relaxations to both thrombin (receptor activated) and A-23187 (not receptor activated) were increased in the 11 mg/day treatment group suggests a general upregulation of calcium-activated nitric oxide synthase activity rather than one associated with selective receptor activation. Alternatively, increases in endothelium-dependent relaxations may reflect changes in the sensitivity of the smooth muscle to nitric oxide. However, observations that all rings of grafts from dogs treated with nicotine-free nicotine-treated dogs contracted to l-NMMA, compared with only 50% of rings with endothelium from control dogs, suggest that nicotine may enhance activity of nitric oxide synthase directly in endothelial cells. Nitric oxide is produced by endothelial cells of reversed saphenous vein CABG in humans (26).

Plasma nitric oxide increased transiently in dogs treated with the 11 mg/day dose after 2–3 wk of treatment. This observation is consistent with sustained increased plasma NOx in human smokers using nicotine nasal spray for smoking cessation (35). Plasma NOx may represent the activity of all three isoenzymes of nitric oxide synthase: neuronal, type I; inducible, type II; or endothelial, type III. All three of these may be affected by nicotine either directly or indirectly. Nicotine stimulates nitroxidergic neurons, leading to the direct release of neurotransmitter nitric oxide. Whether nicotine induces the inducible form of nitric oxide synthase in immunocompetent cells is not known (22). Clearly, definitive pharmacological studies of effects of nicotine on all three isoforms of nitric oxide synthase on transcriptional regulation and enzyme activity are needed. The absence of effect of nicotine on ADP relaxations suggests an agonist specificity for modulation by nicotine.

At first glance, observations of the present study that nicotine treatment may increase endothelium-dependent relaxations may appear to contradict observations that endothelium-dependent relaxations are reduced in human smokers and animals exposed to cigarette smoke (20, 27, 47). However, nicotine is only one of many components of cigarette smoke that could affect endothelial and vascular function (13). This study is the first to examine effects of nicotine treatment on CABG.
bypass grafts independent of smoking. It is important to consider that effects of nicotine may be time and dose dependent. Indeed, acute infusion of nicotine (1–2 μg · kg$^{-1}$ · min$^{-1}$ for 45 min) reduced endothelium-dependent relaxations in microvessels of hamster cheek pouches (30). On the other hand, chronic treatment (0.18–4.7 μg · kg$^{-1}$ · day$^{-1}$ for 2 wk) did not alter endothelium-dependent relaxations to acetylcholine in rat mesenteric arteries (28), but comparable treatment inhibited similar responses in hamster cheek pouch arterioles (31). Differences in responses may reflect species differences in metabolism of nicotine or differences in responsiveness of arteries from various anatomical locations.

In hamster cheek pouch arteries, endothelium-dependent relaxations could be restored by superoxide dismutase (31). This effect suggests that nicotine either directly or indirectly could affect production of oxygen-derived free radicals, which reduce the bioavailability of nitric oxide.

Several differences exist between these former studies and the present one. First, the present study examined endothelium-dependent responses in vein grafts. These conduits had undergone active remodeling during the nicotine treatment. This is in marked contrast to other studies in which effects of nicotine treatment were tested in previously unmanipulated arteries. Also, in the present study, nicotine was administered via transdermal patches; therefore, absorption and metabolic kinetics of the drug would not be the same as delivery by either intraperitoneal injection or osmotic pump (28, 31). For example, with nicotine delivery by osmotic minipumps, circulating concentrations of nicotine would reach a steady state, whereas, with delivery by transdermal patches, circulating concentrations are cyclic peaking at ~6 h after patching and then decaying over the remaining 18 h.

Differences in endothelium-dependent responses with nicotine treatment were expected to show a dose dependency such that increasing doses of nicotine would increase endothelium-dependent responses. This was not the case. On the contrary, with 44 mg/day dosing, endothelium-dependent relaxations were not increased compared with grafts from untreated control dogs. This difference may reflect the multiple sites at which nicotine affects cardiovascular function, includ-

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Table 2. Histological assessment of coronary artery bypass grafts in control dogs and dogs treated with transdermal nicotine

<table>
<thead>
<tr>
<th></th>
<th>Proximal Graft</th>
<th>Mid Graft</th>
<th>Distal Graft</th>
<th>Total</th>
<th>95% CI*</th>
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<tbody>
<tr>
<td></td>
<td>Intimal thickness, μM</td>
<td></td>
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<tr>
<td>Control</td>
<td>118.6 ± 19.3</td>
<td>155.7 ± 33.0</td>
<td>131.7 ± 15.8</td>
<td>135.4 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>11 mg/day nicotine</td>
<td>115.2 ± 24.3</td>
<td>154.6 ± 30.6</td>
<td>182.4 ± 48.3</td>
<td>150.7 ± 24.1 (-38.4 to 69.0)</td>
<td></td>
</tr>
<tr>
<td>44 mg/day nicotine</td>
<td>136.5 ± 53.7</td>
<td>147.8 ± 31.2</td>
<td>127.3 ± 24.6</td>
<td>137.3 ± 20.6 (-43.1 to 46.9)</td>
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|                      | Intimal-to-medial area ratios |           |              |         |         |
| Control              | 0.33 ± 0.07                  | 0.55 ± 0.17 | 0.40 ± 0.03  | 0.42 ± 0.04 |
| 11 mg/day nicotine   | 0.33 ± 0.08                  | 0.49 ± 0.12 | 0.67 ± 0.22  | 0.50 ± 0.12 (-0.18 to 0.34) |
| 44 mg/day nicotine   | 0.44 ± 0.24                  | 0.46 ± 0.12 | 0.48 ± 0.11  | 0.46 ± 0.09 (-0.16 to 0.24) |

Values are means ± SE, n = 4–6/group. See METHODS for calculations. *Confidence interval (95% CI) for difference from control (treatment − control).
ing activation of central and peripheral nicotine receptors (33). Indeed, hemodynamics were not measured during the course of this study. Therefore, it is not possible to determine whether effects of nicotine treatment were direct on endothelial cells or indirect because of changes in hemodynamics due to central autonomic output, which affects blood pressure, flow, and resistance.

Nicotine could stimulate endothelial nitric oxide production indirectly by way of nicotinic stimulation of postganglionic autonomic neurons. Norepinephrine or acetylcholine released from postsynaptic neurons could, in turn, stimulate release of endothelium-derived factors, one of which is nitric oxide (5, 34, 44). Continuous subcutaneous infusion of nicotine in dogs increases release of catecholamines. However, only a
Fig. 7. Representative light micrographs (original magnification ×100) of CABG from an untreated control dog (A) or dogs treated with transdermal nicotine at doses of 11 mg/day (B) or 44 mg/day (C) stained for bone sialoprotein. The intensity of stain was greater in dogs treated with 11 mg/day dose of nicotine, and the stain was distributed into the media compared with grafts from control dogs and dogs treated with 44 mg/day dose nicotine. Arrows denote positive staining.
mild (<10 mmHg) transient hypertension results (3, 18). In humans and nonhuman primates, transdermal delivery also has only minimal hypertensive effects and may even be hypotensive (23, 24), suggesting functional antagonistic actions of nicotine on the cardiovascular system. In addition, nicotine may affect other endothelium-derived factors, some of which may cause vasoconstrictions, as do endothelin and thromboxane (6, 40, 42). In the present study, contractions to α-adrenergic stimulation were reduced in rings with endothelium from the 44 mg/day dose group. The association between nicotine and adrenergic receptors warrants further study.

Another important observation of the present study is that treatment with transdermal nicotine, which achieved serum concentrations higher than those found in humans using nicotine patches for smoking cessation (2, 4), did not adversely affect immediate or subacute (4-wk) graft patency. This observation is consistent with observations in human clinical studies in which using nicotine products for smoking cessation did not increase primary coronary events in high-risk patients (9, 15, 21, 37, 45, 46). Negative effects of nicotine on graft structure might be expected on the basis of experimental literature, because, in cultured endothelial cells, nicotine stimulates proliferation and synthesis of cytoskeletal proteins (6, 10). In subconfluent cultured human arterial smooth muscle cells, cotinine dose-dependently (10$^{-9}$ to 10$^{-6}$ mol/l) augment production of basic fibroblast growth factor, a smooth muscle cell mitogen important to neo-intimal formation, and several matrix metalloproteinases critical to chemotaxis after 12–36 h exposure to nicotine (8). Increases were observed at 10$^{-8}$ and 10$^{-7}$ mol/l of nicotine with inhibition at higher concentrations. These observations suggest that nicotine would affect, in a dose- and time-dependent way, proliferation of smooth muscle cells and matrix secretion. These functions are associated with development of intimal hyperplasia and atherosclerosis, which are the most common causes of subacute and late graft failure. Unexpectedly, no changes in proliferation or neointimal thickness were observed in grafts from nicotine-treated dogs compared with those from control dogs. Differences in proliferation might have been observed if earlier time points were studied (3–7 days when most cell proliferation and migration occurs in vein grafts). Although no differences in cell proliferation were observed in grafts from nicotine-treated dogs, staining for the noncollagenous matrix protein BSP was greater in the media of grafts from dogs treated with the 11 mg/day dose of nicotine. BSP is a highly glycosylated protein that affects integrin interactions associated with cell adhesion to matrix and migration. The phenotype of cells producing BSP relative to other functional characteristics of the cells remains to be determined. However, grafts that expressed greater staining for BSP also showed increased contractions to KCl and relaxations to nitric oxide compared with the other grafts. These results suggest that expression of BSP may be associated with contractile function of smooth muscle, perhaps related to maintenance of membrane potential. Consistent with secretion of metalloproteinase (8), expression of BSP was dependent on but biphasically related to the dose of nicotine, that is, with stimulation of BSP at lower (11 mg/day) compared with higher (44 mg/day) doses.

In summary, results of the present study suggest that treatment with transdermal nicotine affects endothelium-dependent relaxations and secretion of BSP in CABG in dogs. These effects are dependent on the dose of nicotine treatment and may be mediated in part by increased production of nitric oxide and depolarization of the smooth muscle. However, despite these functional changes, transdermal nicotine in doses used for smoking cessation does not appear to decrease patency of reversed saphenous vein CABG in dogs in the immediate 4-wk postoperative period.

There are several limitations that should be considered in extrapolating findings of the present study to humans. First, the number of animals studied was small, and the duration of treatment was short. In addition, the grafts were placed in coronary circulation that was not modified by active disease processes such as smoking or atherosclerosis. Furthermore, metabolism of nicotine may differ between humans and dogs. However, despite these limitations, results indicate that transdermal nicotine treatment in the absence of smoking does not decrease graft patency in the subacute postoperative period. These observations, in conjunction with clinical studies in humans (9, 15, 21, 45), support the conclusion that transdermal nicotine to aid smoking cessation (risk reduction) in patients undergoing bypass grafting does not adversely affect immediate postoperative graft patency.

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