The Effects of Cigarette Smoke and Nicotine on Platelet Thrombus Formation in Stenosed Dog Coronary Arteries: Inhibition with Phentolamine

JOHN D. FOLTS, PH.D., AND FRANK C. BONEBRAKE, B.S., R.N.

SUMMARY  This study was undertaken to examine in vivo the effects of cigarette smoke on cyclic reductions in coronary flow due to platelet thrombus formation in the stenosed coronary arteries of anesthetized dogs. The circumflex coronary artery of 21 mongrel dogs was stenosed 60–80%, with blood flow measured with an electromagnetic flow probe. After the administration of cigarette smoke, plasma epinephrine was elevated nine times the control level (p < 0.001) and peak mean blood pressure was elevated one and one-half times control (p < 0.01). The hematocrit increased several percent (p < 0.01) with cigarette smoke, although blood gases and pH remained unchanged. In all 21 dogs, spontaneous reductions in coronary blood flow were greatly exacerbated in the stenosed circumflex artery as evidenced by the number of flow reductions, the increased size of the reductions and the rate of flow reduction. Nicotine administered intravenously in doses comparable to those achieved through absorption of cigarette smoke by the lungs provoked similar responses of α-adrenergic stimulation and potentiation of the platelet thrombus formation. An α-adrenergic antagonist, phentolamine, was given (3 mg/kg) intravenously to inhibit the exacerbated platelet thrombus formation due to cigarette smoke or infused nicotine. In 18 of 21 dogs, an acute occlusive platelet thrombus was prevented 15 minutes after phentolamine and after a cigarette smoke or nicotine challenge. This study confirms a link between cigarette smoking, platelet thrombus formation, and the potential for humans to develop an acute occlusive platelet thrombus in a diseased and stenotic coronary artery.

CIGARETTE SMOKING has been linked to atherosclerosis since 1908. Other studies have shown cigarette smokers have an increased rate of development of atherosclerosis and increased risk of sudden coronary death and strokes. In recent years, increased platelet activity has been associated with the development of atherosclerotic plaques and has also been implicated in sudden coronary death. Preliminary studies on the effects of cigarette smoke on platelet aggregation in vitro suggest increased platelet activity from a single cigarette. Cigarette smoke increases plasma catecholamines, which in turn exacerbate platelet activity in vivo.

Platelet thrombi collect in stenosed canine coronary arteries, and these thrombi are present in the arterial lumen by histologic section. The periodic appearance of these platelet thrombi causes cyclic reductions in coronary blood flow. In addition, elevating the plasma catecholamine levels exacerbates platelet thrombus formation and the associated cyclic flow reductions.

Therefore, these studies were undertaken to determine if cigarette smoke has a significant effect on the spontaneous cyclic flow reductions observed in dogs given a 60–80% fixed coronary artery stenosis to simulate the condition of coronary artery disease in man. We attempted to ventilate these dogs with cigarette smoke in a manner comparable to human smoking habits.

Materials and Methods

Twenty-one healthy adult mongrel dogs of both sexes were anesthetized with morphine sulfate, 3 mg/kg, followed 30 minutes later by sodium pentobarbital, 30 mg/kg. Respiration was maintained using a Bird pressure cycle respirator. Fixed mechanical obstruction of the circumflex coronary artery was produced using an encircling plastic cylinder as previously described. A 2–0 silk ligature was placed distally around the circumflex to permit temporary occlusion for measuring the hyperemic response, and for checking baseline stability of the electromagnetic flowmeter. A catheter was introduced into the left femoral artery and passed into the proximal aorta for measurement of blood pressure with a Statham-Gould pressure transducer. Another catheter was passed into the distal aorta through the right femoral artery to draw samples for blood gas determinations. Heart rate and ECG were obtained with a wire lead sutured to the pericardium adjacent to the distal circumflex bed. Four-milliliter arterial blood samples were obtained by directly passing an 18-gauge needle connected to a syringe into the left atrium for catecholamine level determinations, as analyzed by Cat-A-Kit. Recording was done with a Brush Gould eight-channel direct-writing recorder.

All dogs were prepared initially in the same way. Two protocols were then carried out.

Group 1 — Administration of Smoke from Cigarettes

A "smoking machine," consisting of a clear polyurethane box that holds a lighted cigarette within the box, is connected to a controlled volume-flow ventilator that delivers a standard volume of cigarette smoke during respiration. The smoke then goes from the burning tip, inside the box, through the tobacco and through tubing to the trachea of the dog. After an observation period of 30 minutes, blood samples were
drawn from 15 dogs for $\text{PO}_2$, $\text{PCO}_2$, pH, hemoglobin, hematocrit and resting plasma catecholamine level determinations. At this time, a standard cigarette, in approximately the middle range as determined by the Federal Trade Commission, April 1976, for tar and nicotine levels (tar 17 mg, nicotine 1.0 mg) was lit and placed in the clear polyurethane box. The endotracheal tube was then switched to a T-valve to the Shields control volume flow ventilator. Through manual cycling of the respirator, a 500-ml volume of room air and cigarette smoke was ventilated into the animal’s lungs, and then the dog was switched back to the Bird respirator. This procedure was repeated approximately twice a minute until the cigarette was finished (approximately 5 minutes). This procedure simulated human smoking patterns. At the conclusion of the cigarette, a second set of blood samples was drawn to detect any changes in blood measurements.

After observing the effect of the cigarette smoke on coronary blood flow for approximately 45 minutes, 3 mg/kg of phentolamine (an $\alpha$-adrenergic antagonist) dissolved in 40 ml of saline was administered over a 5-minute period. Fifteen minutes after the phentolamine was administered, a third set of blood samples was drawn. Using the same brand of cigarette, smoke was delivered to the dog as previously described. At the conclusion of the smoke administration, a fourth set of blood samples was drawn. Smoke was again delivered 45 and 75 minutes after phentolamine administration.

Group 2 — Administration of Intravenous Nicotine

After the 30-minute observation period, a resting blood sample was drawn for catecholamine level determination in six dogs. The dogs were then given 80 $\mu$g/kg of nicotine intravenously through an infusion pump, over 4–5 minutes (corresponding roughly to the period of time it took to ventilate the dog with smoke from one cigarette by our method). When the nicotine infusion was complete, another blood sample was drawn to detect changes in the plasma catecholamine level. Again, the dogs were observed for 45 minutes, and at this time 3 mg/kg of phentolamine was administered as previously described. Fifteen minutes after the phentolamine was administered, a third blood sample was drawn and again 80 $\mu$g/kg of nicotine was infused in the same manner. When the nicotine administration was complete, a fourth blood sample for plasma catecholamine assay was drawn. Nicotine was again administered in the same manner 45 and 75 minutes after phentolamine.

Results

All 21 dogs had spontaneous cyclic reductions of coronary blood flow in the stenosed artery, characterized by slowly falling flow levels with abrupt return to control levels during the 30-minute control period (fig. 1).

Group 1

In all 15 dogs, plasma catecholamine levels and aortic blood pressure increased in response to the administration of smoke from one cigarette (table 1). Arterial $\text{PO}_2$ and $\text{PCO}_2$ did not change significantly, but the hematocrit and hemoglobin levels increased (table 1). In all cases, the spontaneous cyclic flow reductions were more severe, as reflected by the greater flow decrease, the number of flow reductions, and the size of the reductions in flow (table 2). When coronary flow was at or near zero flow and the ECG showed ST-segment deviation suggestive of regional ischemia, the platelet thrombus was dislodged by...
physically shaking the obstructor, restoring control flow. This allowed the experiment to continue. During the 45-minute observation period after cigarette smoke administration, the severity of the exacerbated flow reductions returned to presmoke levels within 5–15 minutes.

After phentolamine, the spontaneous flow reductions were abolished in all dogs. In 13 of the 15 dogs, ventilation with cigarette smoke did not induce any flow reductions in the 15 minutes after phentolamine. In eight dogs, cyclic flow reductions reappeared in response to cigarette smoke 15–75 minutes after phentolamine. In the remaining two dogs, cigarette smoke provoked some platelet thrombus formation within 15 minutes after phentolamine. These flow reductions were less severe than the control reductions in coronary blood flow, and gradually returned to the initial prephentolamine and presmoke levels of severity.

**Group 2**

In all six dogs, i.v. nicotine produced a blood pressure increase and elevation of plasma catecholamines similar to that produced by a cigarette as in group 1 (table 3).

Similar exacerbations of coronary flow reductions were also recorded in response to the nicotine (table 4). Again, as observed in the cigarette smoke trials, in the 45 minutes after the nicotine infusion, the magnitude and frequency of the flow reductions gradually returned to control levels in 5–10 minutes.

After phentolamine, spontaneous flow reductions and those produced by nicotine were abolished in all dogs after 15 minutes. Two of the dogs had a subsequent return of the cyclical flow reductions within 45 minutes during the 75-minute observation period after phentolamine, usually in response to another nicotine challenge.

**Discussion**

With the method we used of stenosing the coronary artery, several of the known factors providing stimuli for platelet aggregation are produced. As the plastic cylinder is placed on the artery, the arterial endothelium is damaged, exposing some collagen, which provokes platelet aggregation. Also, the narrowed lumen probably produces turbulence that could damage platelets and cause some shearing disruption of the red blood cells, thereby liberating ADP, which would provoke platelet aggregation. We and others have shown that spontaneous coronary flow reductions occur in the stenosed coronary artery of our animal model and that this is a result of platelet thrombi formation.

Using this model, an amount of coronary artery stenosis is chosen that just eliminates the reactive hyperemic response to a temporary 20-second complete occlusion. Thus, the coronary flow is in the normal range of 20–40 ml/min before a flow decrease caused by a platelet thrombus beginning to develop. After the thrombus breaks loose, the coronary flow suddenly returns to control levels.

We have also demonstrated that exogenous epinephrine appears to overcome the inhibition of cyclic reductions in coronary flow by aspirin; that is, there is a temporary return of spontaneous reductions of flow. Epinephrine stimulates platelet aggregation in some species and strongly potentiates other aggregating agents in some animals, including the dog.

Nicotine administered i.v. or absorbed from inhaled cigarette smoke raises plasma catecholamines by

**Table 1. Hemodynamic Measurements Before and After the Administration of Cigarette Smoke (n = 15)**

<table>
<thead>
<tr>
<th></th>
<th>ABP (mm Hg)</th>
<th>Po2 (mm Hg)</th>
<th>PCO2 (mm Hg)</th>
<th>pH</th>
<th>Hct</th>
<th>HgB</th>
<th>Plasma epinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cigarette smoke</td>
<td>110 ± 12</td>
<td>97 ± 16</td>
<td>21 ± 6</td>
<td>7.52 ± 0.06</td>
<td>38 ± 4</td>
<td>13 ± 5</td>
<td>206 ± 210</td>
</tr>
<tr>
<td>After cigarette smoke</td>
<td>166 ± 32</td>
<td>97 ± 18</td>
<td>23 ± 6</td>
<td>7.51 ± 0.06</td>
<td>47 ± 6</td>
<td>16 ± 2.0</td>
<td>1817 ± 1188</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviations: ABP = arterial blood pressure; Po2 = partial pressure of oxygen; PCO2 = partial pressure of carbon dioxide; Hct = hematocrit; HgB = hemoglobin.

**Table 2. Quantification of Spontaneous Coronary Blood Flow Reductions in the Circumflex Coronary Artery Before and After the Administration of Cigarette Smoke (n = 15)**

<table>
<thead>
<tr>
<th></th>
<th>Slope of CBF reduction (ml/min²)</th>
<th>Number of CBF reductions/10 min</th>
<th>Size of CBF reductions (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cigarette smoke</td>
<td>-1.75 ± 2.23</td>
<td>1.6 ± 0.9</td>
<td>15.3 ± 13.9</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After cigarette smoke</td>
<td>-10.57 ± 3.48</td>
<td>3.0 ± 1.13</td>
<td>36.3 ± 15.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviation: CBF = coronary blood flow.
directly stimulating the release of epinephrine from the adrenal medulla of dog and man. Our hypothesis is that cigarette smoke would have the effect of exacerbating platelet aggregation in our model, possibly by stimulating the release of endogenous epinephrine or by a direct effect of nicotine on platelets. Epinephrine stimulates platelet aggregation and thrombus formation by an α-adrenergic mechanism. Phentolamine is a good α-adrenergic blocking agent and would thus prevent thrombus formation if the epinephrine stimulus to thrombus formation were the primary mechanisms involved.

We have shown that phentolamine protects against the exacerbation of thrombus formation caused by epinephrine infusions. In our model with "critical" coronary artery stenosis, the circumflex coronary blood flow becomes solely dependent on the perfusion pressure. If arterial pressure increases, coronary flow will increase, until a thrombus forms. As the thrombus gets larger, the flow decreases despite continued elevated blood pressure (fig. 1).

We also obtained blood samples for in vitro platelet aggregation studies before and 5 minutes after the smoking cycle using a previously described aggregometry technique.

We had hoped to show that in vitro platelet activity was increased after smoke, which would have correlated with the increased in vivo platelet activity observed in the animal model. These studies were unsuccessful. The presmoke sample was obtained and processed as previously described. However, the second sample, obtained after the cigarette smoke or nicotine, produced much less platelet-rich plasma. We then determined that the platelets had become so active due to the catecholamine stimulation that during the initial centrifugation to make platelet-rich plasma, the platelets became attached to red cells and also formed platelet aggregates and thus came down with the red cells during centrifugation. Thus, we could not do the usual platelet aggregation study, but demonstrated large platelet aggregates when the centrifuged blood was examined on a peripheral blood smear. We are exploring other methods for demonstrating increased platelet aggregation after smoke or nicotine infusions.

We ventilated our dogs with cigarette smoke over a period that simulates a human smoking pattern. Arterial blood gases and blood pH were not significantly altered by cigarette smoke administration, but plasma epinephrine was significantly elevated (table 1). Concomitant with this increase in plasma epinephrine, blood pressure and hematocrit also increased. The increased hematocrit was probably due to contraction of the spleen, which pools red blood cells in the dog to a greater extent than man.

In a series of kidney transplant experiments in dogs with the spleen in view, we observed that it undergoes a considerable decrease in size with manipulation, or with catecholamine infusions (unpublished observations).

To further elucidate the role of nicotine absorbed from cigarette smoke in these observations, nicotine alone was given intravenously. Blood levels of nicotine obtained by the i.v. injection of 4 μg/kg/min for 20 minutes of nicotine corresponded to the amount absorbed by man during inhalation of one cigarette. The final peak blood levels of nicotine did not depend on whether the nicotine was given over a 20-minute period or in a single injection.

Again, with i.v. nicotine, plasma epinephrine levels and the hematocrit were significantly elevated. Blood pressure increases and equivalent exacerbations of spontaneous flow reductions were noted.

Catecholamine elevation has been observed in male

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**Table 3. Hemodynamic Measurements Before and After an Intravenous Infusion of Nicotine (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>ABP</th>
<th>PO2 (mm Hg)</th>
<th>PCO2 (mm Hg)</th>
<th>pH</th>
<th>Hct</th>
<th>Plasma epinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before i.v. nicotine</td>
<td>118 ± 10</td>
<td>90 ± 18</td>
<td>19 ± 8</td>
<td>7.60 ± 0.08</td>
<td>39 ± 5</td>
<td>315 ± 198</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>After i.v. nicotine</td>
<td>156 ± 28</td>
<td>92 ± 12</td>
<td>24 ± 9</td>
<td>7.52 ± 0.09</td>
<td>44 ± 4</td>
<td>1529 ± 1310</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviations: ABP = arterial blood pressure; PO2 = partial pressure of oxygen; PCO2 = partial pressure of carbon dioxide; Hct = hematocrit.

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**Table 4. Quantification of Spontaneous Coronary Blood Flow Reductions in the Circumflex Coronary Artery Before and After an Intravenous Infusion of Nicotine (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>Slope of CBF reduction (ml/min²)</th>
<th>Number of CBF reductions/10 min</th>
<th>Size of CBF reductions (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before nicotine</td>
<td>-2.11 ± 1.88</td>
<td>1.8 ± 0.8</td>
<td>17.1 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>After nicotine</td>
<td>-8.93 ± 2.98</td>
<td>2.8 ± 2.10</td>
<td>38.1 ± 17.6</td>
</tr>
</tbody>
</table>

(measured for 10 min after administration of nicotine)

Values are mean ± SEM.

Abbreviation: CBF = coronary blood flow.
volunteers after smoking and was associated with an increase in blood pressure and heart rate. Plasma epinephrine increased from 44 ± 4 pg/ml before smoking to 113 ± 27 pg/ml (p < 0.05) 10 minutes after the start of smoking. Plasma norepinephrine increased from 227 ± 23 pg/ml to 324 ± 39 pg/ml (p < 0.05) at 15 minutes after the start of smoking. The smoking also caused an increase in heart rate from 72 ± 3 to 90 ± 2 beats/min (p < 0.001) and mean systolic blood pressure increased from 108 ± 4 to 120 mm Hg (p < 0.01). These hemodynamic changes caused by the catecholamine release due to smoking were blocked by i.v. administration of simultaneous phentolamine and propranolol.

In another study in which pregnant women smoked two cigarettes, plasma epinephrine and norepinephrine were elevated to levels comparable to those reported in the previous study. The increase in plasma catecholamines in the human studies are much less than in our dog model. This may be because of adaptation by the chronic smokers, whereas the dogs had no previous exposure to cigarette smoke.

We did not feel that the bronchial irritation due to the smoke was a major factor, because the dogs were anesthetized; also, we did not observe any significant changes when the nicotine was given intravenously.

Human platelets are believed to have α-adrenergic receptors on their membranes. Stimulation of these receptors can initiate aggregation. If the endogenous epinephrine released in response to the nicotine exacerbated platelet-mediated flow reductions in the stenosed coronary artery, then this should be prevented with an α-adrenergic antagonist. To test this, phentolamine, which has a plasma half-life in anesthetized dogs of approximately 19 minutes, was administered in an attempt to prevent the effect of epinephrine on the platelets. The results suggest that the increased catecholamines caused by smoking probably have a primary effect on the platelets, and thus increase their activity.

Coronary blood flow reductions previously induced by cigarette smoke and the elevated catecholamine levels were prevented by the α-adrenergic antagonist in most dogs. It is not clear why the cyclic flow reductions returned in some of the dogs. With the short half-life of phentolamine, all of the circulating platelets may not have retained an effective α-blockade, permitting some increased platelet aggregation to recur. Some of the dogs may also have had more active platelets than others, and therefore may have required a larger dose of the α-antagonist to maintain effective α-blockade.

We are interested in the fate of these thrombi once they break loose and are carried distally. We have developed a technique for placing a silastic rubber T tube in the circumflex coronary artery distal to the obstruction on the coronary artery. Using this technique, we can catch the thrombi in a beaker of fixative when they break loose. The majority of caught thrombi are obtained intact in a single piece. This allows us to study the ultrastructure microscopically.

This study demonstrates that nicotine, administered intravenously or through the smoke of a cigarette, exacerbates the formation of the platelet-mediated thrombi in stenosed dog coronary arteries.

We plan to study the high- and low-tar and nicotine cigarettes as well as the lettuce-leaf cigarettes, which presumably do not contain nicotine. We are studying the effects of altered hematocrit to determine the effects of high and low hematocrit on platelet thrombus formation in our model.

If this platelet thrombus formation occurs in stenosed human coronary arteries, it is likely to be exacerbated by cigarette smoking and may contribute to sudden coronary death.

Acknowledgment

We thank the cardiology section of the University of Wisconsin Medical School for providing money through the Edward Shovers Memorial Fund for the construction of the smoking machine. Our thanks is also extended to Jeff Anderson, respiratory therapist, for his technical assistance.

References

Effects of Intravenous Prostacyclin in Variant Angina

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A. Distante, M.D., and A. Maseri, M.D.

SUMMARY A lack in prostacyclin (PGI2) production due to atherosclerosis may play a role in the pathophysiology of some of the clinical manifestations of ischemic heart disease and, in particular, of coronary vasospasm. We therefore evaluated the effects of i.v. PGI2 in nine patients with variant angina and six normal volunteers.

In normal subjects, PGI2 (2.5, 5, 10 and 20 μg/kg/min) had significant antiplatelet effects, caused a dose-dependent decrease in both systolic and diastolic arterial pressure and a decrease in pulmonary resistance. Heart rate increased in a dose-dependent manner, but no consistent effects on myocardial contractility (evaluated by ultrasound) were observed. Side effects were negligible and readily reversible.

Although producing obvious antiplatelet and vasodilatory effects, PGI2 did not affect the number, severity and duration of spontaneous ischemic episodes due to coronary vasospasm in five patients and ergonovine-induced spasm in three. However, the number of ischemic episodes was consistently reduced in one patient during four consecutive periods of PGI2 infusion alternated with placebo. A severe, prolonged ischemic episode with ST elevation and pain was consistently observed in this patient every time PGI2 was discontinued.

In the appropriate environment, PGI2 can be administered safely to patients with ischemic heart disease. Occasionally, PGI2 may result in a complete disappearance of ischemic episodes due to coronary vasospasm, but usually it is ineffective. These conflicting results could be related to different etiologies of coronary spasm.

PROSTACYCLIN (PGI2), an arachidonic acid metabolite produced by the vascular endothelial cells and by the lungs,14 exerts powerful vasodilating and antiplatelet effects in vitro9,10 and in vivo.11-12 These effects may avoid intravascular thrombosis, maintain endothelial integrity and, possibly, control vascular smooth muscle tone. Decreased PGI2 production by the atherosclerotic arterial wall might play a role in some of the clinical manifestations of ischemic heart disease.13,18-20 Maseri et al.21 suggested that coronary vasospasm could result from an increased vascular sensitivity to vasoconstrictor stimuli, secondary to a reduction in local PGI2 production at the site of atherosclerotic lesions. Moreover, the hypothesis that transient coronary vasospasm might be precipitated by thromboxane A2, locally released by aggregating platelets,22 in the presence of endothelial lesions has recently gained attention.23-26 Finally, cyclical transient reduction in coronary flow, occurring in experimentally narrowed coronary arteries,28-29 has been prevented by the administration of PGI2.

In a preliminary study, we investigated the hemodynamic, antiplatelet and possible side effects of PGI2 in six healthy volunteers. In a second study, we evaluated the effects of PGI2 infusion in nine patients with variant angina, traditionally considered a clinical landmark of transient coronary vasospasm.29-33

Material and Methods

Study 1

Subjects

Five healthy male subjects, all coauthors of the present report (SC, GC, GAC, AM and CP), ages 32-50 years (mean age 38 years) volunteered for the study. One male patient (NV), age 34 years, submitted to routine coronary angiography and coronary sinus catheterization because of atypical precordial pain. He gave informed consent to the infusion of the drug after the completion of the diagnostic study, which had failed to demonstrate coronary lesions or hemodynamic or myocardial metabolic abnormality.

PGI2 Preparation and Mode of Administration

The sodium salt of PGI2 (Wellcome Laboratories) was dissolved in glycine buffer (pH 10) and injected, by a constant-flow Harvard pump, into a right ante-
The effects of cigarette smoke and nicotine on platelet thrombus formation in stenosed dog coronary arteries: inhibition with phentolamine.

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