Oral Verapamil Inhibits Platelet Thrombus Formation in Humans

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Background Calcium antagonists such as verapamil are potent coronary and systemic vasodilators that are used in the treatment of coronary disease. They have also been shown to inhibit platelet aggregation in vitro, but whether they have beneficial antithrombotic effects in humans is unclear, and whether they can potentiate the antithrombotic effects of aspirin is unknown.

Methods and Results Platelet thrombus formation and whole blood platelet aggregation were measured in 18 stable coronary patients on three separate occasions: at baseline when receiving no active medications, after 7 days of receiving oral verapamil SR (240 mg/d), and after 7 days of receiving a combination of oral verapamil SR and aspirin (325 mg/d). Thrombus formation on porcine aortic media that were placed into cylindrical flow chambers and exposed to flowing antecubital venous blood for 3 minutes was assessed morphometrically at a shear rate of 2546 s⁻¹, which is typical of arterial flow at sites of stenoses. Thrombus formation under basal conditions was 7.0±1.6 μm², and this was decreased to 3.1±0.5 μm² (P<.05) after 7 days of treatment with oral verapamil SR and to 2.6±0.5 μm² (P<.05) after 7 days of treatment with oral verapamil and aspirin. Whole blood platelet aggregation levels in response to 0.050 and 0.075 U of thrombin at baseline were 10.8±1.0 and 11.9±1.0 Ω; aggregation was inhibited after 7 days of treatment with verapamil to 6.5±1.1 and 7.8±0.9 Ω (P<.05 versus baseline) and after 7 days of treatment with verapamil and aspirin to 6.1±1.1 and 7.2±1.0 Ω (P<.05), respectively.

Conclusions The present study demonstrates that part of the benefit of verapamil in ischemic heart disease may occur by inhibition of platelet aggregation and thrombus formation. Thus, beneficial antithrombotic effect may be important in preventing acute coronary ischemic events resulting from thrombus formation at sites of plaque rupture.

Key Words • verapamil • platelets • thrombosis • coronary disease

Verapamil is a calcium channel blocker that has potent coronary and systemic vasodilator properties and is used in the management of patients with coronary artery disease.¹⁻⁸ These effects of verapamil are mediated through inhibition of the calcium fluxes across plasma membranes.¹⁻³ Several platelet functions are also dependent on intracellular calcium for optimal activation.⁹⁻¹² and by inhibiting calcium fluxes and its intracellular messenger role in platelets, verapamil has been shown to inhibit platelet aggregation and platelet deposition on vascular grafts in vivo.¹³⁻¹⁷ However, demonstration of the inhibition of platelet aggregation ex vivo and in vitro requires a higher concentration of calcium channel blockers (25 to 500 μmol/L)¹⁴⁻¹⁷ than can be achieved at clinically used doses; peak verapamil blood levels range from 0.1 to 1.0 μmol/L.¹⁸ This discrepancy has raised questions regarding the significance of inhibition of platelet function by the calcium channel blockers, including verapamil.

Platelet activation, thromboxane A₂ generation, and platelet thrombus formation play an important role in the precipitation of the acute ischemic coronary syndromes,¹⁹,²⁰ in which plaque rupture is a common underlying pathological event in the evolution of the atherosclerotic plaque.²¹ In these syndromes, thrombox-

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Evaluation of Platelet and Thrombus Formation

A 19-gauge butterfly cannula was inserted atraumatically into an antecubital vein, and the flowing venous blood from the patient was drawn over porcine aortic media held in Plexiglas superfusion flow chambers23-25 with the use of a peristaltic pump (model 701A, Masterflex, Cole-Farmer Instruments Co, Chicago, Ill) placed distal to the chambers. These chambers were designed to mimic the tubelike shape of the vascular system23-25 and contained a window that permitted direct exposure of the aortic media to the flowing venous blood, which was then discarded after its passage through the chambers. A 3-minute superfusion of the aortic media was performed at a shear rate of 2546 s⁻¹ with the flow chambers maintained at 37°C in a water bath. The aortic media used in the superfusion chambers were obtained from normal pig aortas by opening the aorta longitudinally and peeling off and discarding the intima and the thin portion of the subjacent media. The remaining aortic media were then divided into 35×15-mm segments to be placed inside the superfusion flow chambers so that the aortic media were exposed to flowing blood in the chamber.23-25 Exposure of the arterial media simulates a type 3 arterial wall injury with a thrombogenic response, like that seen with plaque rupture.22

After the superfusion, the aortic media strips were removed from the chambers, fixed in 10% Formalin, and processed for histological analysis. Vertical cross sections were made in the proximal, mid, and distal third portions of each strip and stained with hematoxylin phloxin safran (Fig 1). The stained histological tissue was then analyzed under a light microscope (model Diapan, Leitz Co, Toronto, Ontario, Canada), and platelet thrombus formation on the aortic media was quantitated morphometrically (in μm²) by viewing the thrombus mass through the microscope at 100× magnification and tracing the outline using a side-tube attachment to the microscope. The outlined length was then measured with a digitizing tablet and an IBM-AT-compatible computer.

All measurements were made by one of the authors, who was blinded to the treatment the patients received. Thrombus size measurements were expressed as the average of nine analyzed sections per tissue (three in the proximal, three in the mid, and three in the distal section), expressed as the surface area in microns squared. This morphometric analysis was validated and shows a strong correlation (r = .84, P = .0001) between the amount of 111In-labeled platelets deposited on the media and the morphometrically assessed thrombus size (Fig 1). There is also excellent reproducibility between measurements performed 1 week apart and a good correlation between measurements 1 and 2 (r = .95, P = .0001).

Platelet Aggregation Studies

In vitro whole blood platelet aggregation was performed using an impedance aggregometer26-27 (Chronolog Corp, Havertown, Pa) and fresh venous blood. After a 1:1 dilution of native blood with normal saline, the aggregation was induced by adding 50 μL of the aggregating agent thrombin, 0.050 or 0.075 National Institutes of Health unit/mL (Hoechst Behring, France). All studies were performed within the first minute after blood sampling. Platelet aggregation was automatically quantified (amplitude ± 3 minutes after addition of the aggregation agents with AGGRO/LINK software (Chronolog Corp).

Data Analysis

Multiple comparisons were made by ANOVA, and when significant, pairwise comparison was made by Dunnett’s test. A value of P < .05 was considered significant.

Results

Blood Pressure, Heart Rate, and Hematological Variables

The variations of mean arterial blood pressure and heart rate for each of the three study periods (baseline, verapamil, and verapamil plus aspirin) are shown in the Table. After 1 week of treatment with verapamil SR, patients’ arterial blood pressure and heart rate decreased significantly. Relative to baseline, blood pressure and heart rate remained low during the second week of treatment when aspirin was added, but there was no significant difference between the values for patients receiving verapamil and for those receiving the combination of verapamil and aspirin. The medications were well tolerated, with one patient reporting mild constipation, which did not require drug modification or withdrawal, over the 2-week study period. Blood platelet count was slightly higher after treatment with verapamil, but hematocrit remained stable. There were no significant differences in the antithrombotic responses between men and women or between those who had had a previous myocardial infarction and those who had not.
Platelet Thrombus Formation

The decrease in blood pressure did not influence the assessment of platelet thrombus formation because the perfusion studies were conducted under constant flow and with shear rate regulated by the peristaltic pump. Mural platelet thrombus formation on the perfused arterial wall media was readily appreciated under the microscope at 100x magnification, as shown by a typical example in Fig 3. After 1 week of verapamil therapy, less thrombi formed than had at baseline. The extent of platelet thrombus formation, as assessed morphometrically (in μm²), was reduced 56% by verapamil compared with baseline (P<.05), as shown in Fig 4, where baseline thrombus size was 7.0±1.6 μm². The combination of verapamil and aspirin reduced thrombus size by 63% relative to baseline (P<.05), but the addition of aspirin did not significantly contribute additional antithrombotic effect (P=NS) as did verapamil alone (Fig 4).

Platelet Aggregation

Immediately after samples of blood were taken, platelet aggregation in whole blood in response to each dose of thrombin was significantly reduced by both verapamil alone and by the combination of verapamil and aspirin, as shown in the Table. Similar to what is observed with the inhibition of mural platelet thrombus formation on the aortic media, the addition of aspirin did not potentiate the platelet-inhibitory effect of verapamil observed in the aggregometer.

Discussion

This study demonstrates for the first time that at clinical doses, in patients with stable angina oral long-acting verapamil significantly inhibits platelet thrombus formation on the thrombogenic arterial wall media exposed to circulating blood. In addition, whole blood platelet aggregation in response to thrombin is significantly inhibited. This inhibition of platelet aggregation and platelet-vessel wall interaction leading to less mural thrombus formation was not potentiated by the concomitant use of aspirin. These effects could be observed after 7 days of treatment with oral verapamil and were associated with a significant reduction in blood pressure and heart rate. It is possible that these beneficial antithrombotic effects may be valuable in reducing long-term ischemic cardiac events in patients.
with coronary disease and provide antithrombotic protection for those unable to take aspirin for secondary prevention.

**Antiplatelet and Antithrombotic Effects**

As a class of agents, calcium channel blockers have been shown to inhibit human platelet aggregation in response to several agonists in platelet-rich plasma, but the concentrations required to inhibit platelet aggregation in vitro generally have been high, much higher than those achieved after conventional oral dosing. The relevance of the antiplatelet effects of calcium channel blockers has thus been questioned. However, the methods used in previous studies may not accurately reflect conditions in the intact circulation. The time-consuming and extensive steps involved in preparing platelet-rich plasma may modify platelet function and deplete short half-life mediators (such as endothelium-derived relaxing factor, prostacyclin, and thromboxane A2), all of which can modulate the response of platelet-rich plasma to calcium channel blockers. Other blood components, such as red blood cells and neutrophils, that affect platelet behavior also are excluded from the testing milieu of the platelet-rich plasma. Determination of platelet aggregation in whole blood at the bedside within 1 minute of sampling minimizes many of the above limitations associated with aggregation in platelet-rich plasma and can yield different results, which may be more relevant to the in vivo situation.

With the whole blood aggregation technique, oral verapamil at a usual dose exerted significant platelet-inhibitory effects in this study.

Nevertheless, inhibition of platelet aggregation in vitro and ex vivo may not necessarily correlate with clinically relevant antithrombotic properties. Even platelet aggregation in whole blood may not adequately reflect in vivo platelet activation at sites of stenosis and plaque rupture because the effects of shear forces and arterial wall components are not examined during specific agonist-induced platelet aggregation in vitro. To partially overcome this situation, we assessed the effect of verapamil in a model that simulates plaque rupture. The platelet-inhibitory effects of verapamil may be clinically relevant because the drug significantly inhibited platelet thrombus formation in this model. It is of importance that this antithrombotic property was observed at a high shear rate—2546 s⁻¹—as typically occurs at sites of arterial stenoses.

**Mechanism of Action**

Many of the processes involved in the platelet–vessel wall interactions, including the adherence and subsequent platelet aggregation and release, are calcium dependent. Increase in intracellular ionized calcium is believed to act as a key second messenger in platelet function, and calcium channel blockers appear to inhibit platelet function by interfering with these transmembrane calcium fluxes.

Calcium channel blockers have also been shown to inhibit platelet function by an anesthetic-like effect on cell membranes, to inhibit thromboxane A2 formation, and to increase intracellular cAMP by inhibiting cAMP phosphodiesterase. Inhibition of platelet response to thromboxane A2 by verapamil would produce an effect similar to that induced by aspirin, and this may explain why the antiplatelet and antithrombotic effects noted in this study were not potentiated by aspirin. In addition, verapamil may inhibit a more distal step involved in platelet aggregation than the prostaglandin pathway. Recently, it has been shown that verapamil can interfere with the platelet glycoprotein Ib/IIIa receptor complex, which is involved in mediating calcium fluxes as well as being the final common step involved in platelet aggregation and platelet thrombus formation.

**Clinical Relevance**

The introduction of calcium channel blockers has been an advance in the medical management of patients with coronary artery disease. Agents like verapamil reduce blood pressure and heart rate, prolong exercise tolerance and reduce exercise-induced myocardial ischemia, suppress coronary vasospasm, and ameliorate patients with Prinzmetal's angina. Verapamil also prevents reinfarction after an episode of myocardial infarction and is effective in patients with unstable angina, syndromes where coronary thrombosis and release of thromboxane A2 play an important precipitating role. The ability of verapamil to block calcium influx into cells and the intracellular messenger role of calcium are probably responsible for the cardiac and vascular effects of verapamil. Its beneficial effects in acute ischemic coronary syndromes may reflect an additional antithrombotic property at clinically relevant doses, as shown in the present study. It has even been suggested that the antianginal efficacy of verapamil may be related in part to its platelet-inhibitory action.

Because verapamil also is often used to lower blood pressure, this antithrombotic effect might contribute to a decrease in the secondary complications of myocardial infarction and stroke in hypertensive patients, complications that are responsive to aspirin.

**Conclusions**

Orally administrated verapamil exerts potent antiplatelet and antithrombotic effects in stable coronary patients. This antiplatelet effect may provide beneficial antithrombotic coverage in addition to known vascular and hemodynamic properties that could be advanta-
geous in the management of coronary patients and the prevention of ischemic coronary events resulting from acute thrombus formation. Verapamil also inhibited thrombin-induced platelet aggregation, an advantage that aspirin does not possess.

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