Antithrombotic Efficacy of the Vitamin K Antagonist Fluindione in a Human Ex Vivo Model of Arterial Thrombosis

Effect of Anticoagulation Level and Combination Therapy With Aspirin

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Abstract—Thrombin is a main mediator of arterial thrombus formation, and its inhibition is an important antithrombotic strategy. However, the place of vitamin K antagonists among the different therapeutic strategies for preventing arterial thrombus formation is still debated. We studied the antithrombotic efficacy of the vitamin K antagonist fluindione in a human ex vivo model of arterial thrombosis and determined whether aspirin enhances fluindione efficacy. Ten healthy male volunteers were randomly assigned to receive fluindione, alone or in combination with aspirin (325 mg/d). Fluindione was given at increasing doses to give a stable international normalized ratio (INR) between 1.5 and 2.0 and between 2.1 and 3.0. We induced arterial thrombus formation ex vivo by exposing collagen- or tissue factor (TF)–coated coverslips in a parallel-plate perfusion chamber to native blood for 3 minutes at an arterial wall shear rate of 2600 s⁻¹. Platelet and fibrin deposition were measured by immunoenzymatic methods. Fluindione inhibited thrombus formation on TF-coated coverslips in a dose-dependent manner by 50% and 80% at INR 1.5 to 2.0 and INR 2.1 to 3.0, respectively (P<0.05). Fluindione in combination with aspirin inhibited TF-induced thrombus formation in a comparable manner. Collagen-induced thrombus formation was not reduced in subjects treated by fluindione. It was reduced by 50% to 60% in those treated with fluindione plus aspirin, regardless of the level of anticoagulation (P<0.05). Thus, the effectiveness of fluindione for preventing arterial thrombosis is dependent on the nature of the thrombogenic trigger. Fluindione is very effective in preventing TF- but not collagen-triggered thrombus formation. Aspirin enhances the antithrombotic effectiveness of fluindione, because combined treatment interrupts both TF- and collagen-induced thrombus formation.


Key Words: thrombosis ■ aspirin ■ anticoagulants

Oral vitamin K antagonists are effective and widely used agents for the treatment of venous thrombosis and the prevention of systemic embolism in patients with prosthetic heart valves and atrial fibrillation. However, the place of vitamin K antagonists among the different therapeutic strategies for preventing arterial thrombosis is still debated, despite the fact that thrombin is a primary mediator of arterial thrombus formation and that inhibition of thrombin generation is an important arterial antithrombotic strategy. Few studies suggest that vitamin K antagonists are useful in this setting, but information regarding the effectiveness of this therapy and the relationship between the level of anticoagulation as expressed by the international normalized ratio (INR) and the antithrombotic effect is still lacking.

Aspirin is widely used for the prevention and treatment of acute and chronic arterial diseases, but it has moderate clinical efficacy. Because the pathogenesis of thrombosis is complex and multifactorial, a combination of anticoagulant and antiplatelet therapy represents an attractive alternative strategy. Some studies have shown the clinical benefit of such combined treatment among mechanical heart valve recipients. Data regarding acute ischemic heart disease are more controversial. Thus, the important questions that remain are whether the combination of aspirin and oral anticoagulant therapy improves the antithrombotic efficacy of either drug alone in the setting of arterial thrombosis and whether this efficacy appears at low or high levels of anticoagulation.

The antithrombotic effect of drugs can be experimentally investigated in humans by use of an ex vivo model of thrombogenesis that closely mimics relevant clinical situations. In this model, native blood is drawn from volunteers through a parallel-plate chamber device, where it interacts with a thrombogenic surface in well-established blood flow conditions, mimicking wall shear rates encountered in
moderately stenosed arteries (2600 s⁻¹). Two relevant thrombogenic molecules, collagen and tissue factor (TF), which are present in atherosclerotic plaques and primarily responsible for thrombus formation in vivo, are exposed to blood. The efficacy of antithrombotic drugs is determined by quantification of the respective thrombus content in platelets and fibrin by immunoenzymatic methods. This model has been used to investigate numerous antithrombotic strategies, and results appear consistent with clinical data.

In the present study, we used this ex vivo model of acute initial arterial thrombus formation to determine the efficacy of the vitamin K antagonist fluindione in preventing arterial thrombosis, the level of anticoagulation required to obtain an antithrombotic effect, and whether fluindione in combination with aspirin improves the antithrombotic efficacy of fluindione alone. We designed an open, randomized study in healthy male volunteers. Fluindione was administered either alone or in combination with aspirin, and it was given to obtain a low (INR 1.5 to 2.0) and a conventional (INR 2.1 to 3.0) level of anticoagulation, respectively.

Methods

Subjects

The study population consisted of 10 healthy white male volunteers aged 18 to 40 years. They had no history or clinical sign of any disease and did not take any medication known to affect blood coagulation or platelets during the study period. The volunteers smoked <10 cigarettes per day, and they did not smoke on the day of the perfusion experiments. Clinical chemistry, hematological, and hemostatic laboratory values were within the normal ranges. All subjects gave written informed consent to the protocol, which was approved by the local Human Subjects Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Toulouse, France).

Study Design

This monocentric, randomized controlled study was performed in the Center for Clinical Investigation at Hôpital Purpan, Toulouse, France. After selection for the trial, each volunteer followed 3 sessions, in random order, in which either no treatment, fluindione alone (Procter and Gamble Pharmaceuticals), or combined fluindione plus aspirin (325 mg/d, UPSA) was given. These 3 sessions were separated by a washout period of 3 to 6 weeks. For the control session, volunteers performed only the blood donation for experimental thrombogenesis. For the fluindione sessions, blood donation was performed when INR was stable within the respective target ranges, i.e., 1.5 to 2.0 and 2.1 to 3.0. The initial dose of fluindione was set at 15 mg/d. INR was measured 3 times per week, and dosage was adjusted until INR had stabilized first between 1.5 and 2.0 and then between 2.1 and 3.0 for ≥3 consecutive days. In the fluindione plus aspirin session, aspirin treatment was initiated on the first day fluindione was given. A clinical follow-up was done on each visit at the study center, and at the end of each period of treatment, 10 mg of vitamin K was administered to reverse the anticoagulant treatment. All adverse effects were recorded, and appropriate follow-ups were scheduled.

Preparation of Thrombogenic Surfaces

The thrombogenic molecules were coated on Thermanox plastic coverslips (Miles Laboratories) as previously described. Equine collagen (Collagen Reagent Horn, Nycomed) was sprayed onto plastic coverslips to a final density of 0.5 μg/cm². TF, purified from human placenta (Thromborel, Behring), was diluted 1:133 in coating buffer (0.1 mol/L sodium carbonate, pH 9.5), and coverslips were incubated in 2 mL of the Thromborel dilution for 24 hours at 4°C.

Perfusion Experiments

Two perfusion experiments were performed after each period of treatment, with collagen and TF as the thrombogenic surface, respectively. Perfusion experiments were performed with a parallel-plate perfusion chamber device at 37°C. After blood samples were collected (see below), native blood was drawn directly from an antecubital vein of the volunteers through a 19-gauge infusion set (Ohmeda) over a collagen-coated coverslip positioned in the parallel-plate perfusion chamber. The blood flow rate was maintained at 10 mL/min by a peristaltic roller pump (Multiperx LKB, Pharmacia) placed distal to the chamber. The wall shear rate at the thrombogenic surface was 2600 s⁻¹. The blood perfusion experiment lasted for 3 minutes and was followed by a 30-second perfusion of PBS to wash out blood from the flow channel. The accumulated thrombotic deposits were removed from the chamber and divided into 2 equal parts parallel to the direction of the blood flow, as described previously. One half was placed in a plasmin solution and processed as described below. The other half was immersed into freshly prepared fixation solution (2.5% glutaraldehyde in 0.1 mol/L cacodylate, pH 7.4) at 4°C for 90 minutes and stored in 0.1 mol/L cacodylate/7% sucrose at 4°C until embedded in epoxy resin. A second perfusion experiment was subsequently performed with blood drawn directly from a contralateral cubital vein over a TF-coated coverslip.

Determination of Thrombus Formation

Immunological Determination of Fibrin and Platelet Deposition

Fibrin deposition was quantified by immunological determination of fibrin-degradation products of plasmin-digested thrombi, as described previously. After perfusion, the thrombus was immediately incubated in 2 mL of a plasmin solution (Chromogenix; 0.7 U/mL in Tris-buffered saline, pH 7.4) for 30 minutes under gentle shaking at 37°C. Plasmin digestion was stopped by the addition of aprotinin (2000 KIU/mL, Sandoz). The solution was centrifuged (4°C, 4300 g, 15 minutes) and the supernatant frozen at −80°C for measurement of fibrin-degradation products and P-selectin levels (see below). Fibrin-degradation products were measured by an immunoenzymatic assay (Asserachrom D-Di, Stago). The amount of deposited fibrin was determined directly from the levels of fibrin-degradation products expressed in fibrin equivalent units as indicated by the manufacturer.

Platelet deposition was quantified by measurement of a specific platelet α-granule membrane protein, P-selectin. After centrifugation of the plasmin-digested thrombus, the pellet was dissolved in 400 μL of a lytic buffer, frozen and thawed 3 times, and then sonicated (4°C, 20 kHz) for 270 seconds. The lytic buffer was centrifuged (10,000 rpm, 4°C, 20 minutes) and the supernatant frozen at −80°C for measurement of fibrin-degradation products and P-selectin levels (see below). The amount of deposited fibrin was determined directly from the levels of fibrin-degradation products expressed in fibrin equivalent units as indicated by the manufacturer.

Morphometric Determination of Platelet Adhesion

Microscopic evaluation of platelet adhesion was performed on epoxy-embedded semithin sections (1 μm thick) stained with toluidine blue and basic fuchsins, as previously described. The sections were prepared at an axial position of 2 mm downstream from the upstream edge of the coverslip and perpendicular to the direction of the blood flow. Standard morphometry, performed by light microscopy at 1000× magnification, was used to quantify the percent coverage with fibrin recognized as circular spots associated or not associated with platelets (percent fibrin deposition) and with platelets adherent to collagen or fibrin (percent platelet adhesion). We performed these morphometric evaluations at 10-μm intervals
Along the surface by moving the section along an eyepiece micrometer in the microscope ocular.

**Determination of Platelet Activation and Thrombin Formation**

Platelet activation and thrombin generation were determined by measurement of plasma levels of β-thromboglobulin (βTG) and thrombin-antithrombin complexes (T-AT), respectively. βTG and T-AT were measured in blood (3.2 mL) collected in 0°C precooled syringes containing a mixture (0.8 mL) of platelet inhibitors and anticoagulants (sodium citrate, citric acid, theophylline, adenosine, dipyridamole, heparin, and aprotinin), as described previously. Blood samples were collected at the flow outlet of the chambers between 2.5 and 3 minutes of perfusion by a syringe pump (Harvard Apparatus). Blood samples were immediately centrifuged (4300 g, 4°C, 30 minutes), and aliquots of plasma were stored at −80°C until assayed. Plasma concentrations of βTG and T-AT were measured by immunoenzymatic assays (Assera-βTG, Stago, and Enzygnost-T-AT, Behring, respectively).

**Other Laboratory Procedures**

Red cell, leukocyte, and platelet counts, hemoglobin, and hematocrit were measured by an electronic counting device (model S plus, Coulter Electronics) during and after each period of treatment. Coagulation times were determined with fresh blood samples collected in citrated evacuated container tubes (0.5 mL of 0.129 mol/L trisodium citrate for 4.5 mL of blood; Becton Dickinson) by use of a coagulometer (STA, Stago). Prothrombin times (PTs) were measured with Tromborel (Behring; international sensitivity index [ISI] 1.03) and expressed as INRs according to the formula INR = (patient PT/control PT)1.0. One-stage assays of factors II, VII, and X were performed with plasmas deficient in factor II (Biomeérieux), VII (Stago), or X (Stago), respectively. One-stage assays of coagulation factor IX were performed with automated APTT (Organon Teknika) and factor IX–deficient plasmas (Behring). We checked subjects’ compliance to aspirin treatment by performing platelet aggregation tests with arachidonic acid (1 mmol/L final concentration; BioData Corporation) and a platelet aggregometer (Coulter Electronics).

**Statistical Analysis**

Statistical analyses were performed with the PCSM program (Delta-soft). Voluntees were randomized according to a 3-treatment, 3-period crossover design. Results were expressed as mean ± SEM. Statistical comparison was performed with an ANOVA followed by a Neuman-Keuls test when the P value was ≤0.05. In comparisons of 2 groups, probability values were calculated by the Wilcoxon test.

**Results**

**Study Population**

Ten white male subjects aged 22 to 31 years (mean age 24 years) were enrolled in the study and randomly assigned to the different treatments from January 1997 through January 1998. None prematurely stopped the trial. Minor adverse events, notably bleeding and gastrointestinal disorders, were rare and moderate and did not lead to interruption of treatment. One small cutaneous hematoma was observed in each of 2 subjects with a conventional anticoagulation level of fluindione combined with aspirin.

**Blood Parameters**

Table 1 summarizes the mean doses and durations of fluindione treatment required to obtain INRs within the target range. The respective target INRs, ie, 1.5 to 2.0 and 2.1 to 3.0, were obtained in all patients (Table 1) except 1 who received fluindione plus aspirin therapy, in whom INR was 3.2 when blood donation for experimental thrombogenesis was performed. Oral anticoagulant treatment led to a decline in the activity of factors II, VII, IX, and X that was dependent on the level of anticoagulation. All these parameters were comparable, even with the combination of aspirin and fluindione (P = NS). Finally, platelet aggregation to arachidonic acid was fully inhibited (≥90%) in all 10 volunteers given aspirin plus fluindione, indicating good compliance with aspirin treatment.

**Effect of Treatment on TF-Induced Thrombus Formation**

Thrombi formed on TF-coated coverslips in control experiments were rich in fibrin and platelets, with platelets deposited almost exclusively on top of fibrin meshes. Fluindione inhibited platelet and fibrin deposition, as measured by immunoenzymatic methods (Figure 1), in a dose-dependent manner. At low anticoagulation levels (INR 1.5 to 2.0), platelet and fibrin deposition were prevented by 50% and 55%, respectively (P < 0.05 versus control). Conventional fluindione treatment (INR 2.1 to 3.0) reduced platelet and fibrin deposition by 78% and

<p>| Table 1. Dosage, Duration, and Effect on Coagulation of Fluindione Regimens |
|-----------------------------|-----------------------------|-----------------------------|
|                            | Control                     | Fluindione                  | Fluindione + Aspirin          |</p>
<table>
<thead>
<tr>
<th></th>
<th>INR</th>
<th>INR</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mg</td>
<td>None</td>
<td>1.5–2.0</td>
<td>2.1–3.0</td>
</tr>
<tr>
<td>Duration, d</td>
<td>...</td>
<td>20.5±2.4</td>
<td>27.8±2.3</td>
</tr>
<tr>
<td>PT, s</td>
<td>...</td>
<td>12.8±0.2</td>
<td>20.7±0.6</td>
</tr>
<tr>
<td>INR</td>
<td>...</td>
<td>1.7±0.1</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>Factor II, %</td>
<td>103±4</td>
<td>43±3</td>
<td>28±2</td>
</tr>
<tr>
<td>Factor VII, %</td>
<td>93±7</td>
<td>43±4</td>
<td>27±3</td>
</tr>
<tr>
<td>Factor X, %</td>
<td>112±5</td>
<td>36±2</td>
<td>21±2</td>
</tr>
<tr>
<td>Factor IX, %</td>
<td>100±5</td>
<td>53±5</td>
<td>34±3</td>
</tr>
</tbody>
</table>

Fluindione, alone or combined with aspirin, was given to 10 healthy volunteers to obtain respective target INRs. Blood for measurement of prothrombin times (PT), INR, and % activity of plasma coagulation factors was collected just before blood donation for experimental thrombogenesis.
84%, which was significantly more potent than the lower fluindione anticoagulant treatment ($P<0.02$). When aspirin was combined with fluindione, platelet and fibrin deposition were reduced in a dose-dependent manner comparable to that obtained with fluindione alone (Figure 1).

Morphometric analysis confirmed that fibrin deposition and thus platelet adhesion to fibrin meshes were reduced in a dose-dependent manner by fluindione (Table 2). However, in subjects treated with the combination of aspirin and conventional-intensity fluindione, platelet adhesion to fibrin meshes was significantly more reduced than in those treated with fluindione alone ($P<0.05$; Table 2).

Fluindione also prevented $\beta$-TG release and T-AT formation in a dose-dependent manner ($P<0.05$ versus control; Figure 2). A comparable effect was found in subjects treated with the combination of aspirin and fluindione. The antithrombotic effect in the presence or absence of aspirin was significantly correlated to INR and to the plasma levels of factors X and II but not of factors VII and IX (Table 2).

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**Table 2. Effect of Fluindione Regimens on Fibrin Deposition and Platelet Adhesion Measured by Morphometry**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fluindione</th>
<th>Fluindione + Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>INR 1.5</td>
<td>INR 2.5</td>
</tr>
<tr>
<td>TF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet adhesion, %</td>
<td>34.4±2.8</td>
<td>19.1±5.3*</td>
<td>11.5±4.8†</td>
</tr>
<tr>
<td>Fibrin deposition, %</td>
<td>57.4±6.3</td>
<td>33.9±10.2</td>
<td>20.0±9.6*</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet adhesion, %</td>
<td>47.7±2.1</td>
<td>45.7±3.9</td>
<td>47.2±3.9</td>
</tr>
<tr>
<td>Fibrin deposition, %</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* $P<0.05$, † $P<0.01$ vs control.

Platelet adhesion and fibrin deposition represent the percent coverage with platelets adherent to collagen or fibrin (% platelets) and with fibrin associated or not associated with platelets (% fibrin) after 3 minutes perfusion at 2600 s$^{-1}$.

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Figure 1. Effect of different fluindione regimens on deposition of platelets and fibrin on TF-coated coverslips. The surface was exposed for 3 minutes to native blood at a shear rate of 2600 s$^{-1}$ from volunteers given either fluindione (open bars) or the combination of fluindione plus aspirin (ASA; hatched bars). Target INRs were 1.5 to 2.0 (INR1.5) and 2.1 to 3.0 (INR2.5). No treatment was given during the control period (solid bars). Values are mean±SEM. *$P<0.05$, **$P<0.01$, fluindione or fluindione plus aspirin vs control.

Figure 2. Effect of different fluindione regimens on activation of platelets and coagulation by thrombus formation triggered by TF-coated coverslips. The surface was exposed for 3 minutes to native blood at a shear rate of 2600 s$^{-1}$ from volunteers given either fluindione (open bars) or the combination of fluindione plus aspirin (ASA; hatched bars). Target INRs were 1.5 to 2.0 (INR1.5) and 2.1 to 3.0 (INR2.5). No treatment was given during the control period (solid bars). Markers of activation of platelets ($\beta$-TG) and formation of thrombin (T-AT) were measured in blood collected at the flow outlet of the chamber between 2.5 and 3 minutes' perfusion. Values are mean±SEM. *$P<0.05$, **$P<0.01$, fluindione or fluindione plus aspirin vs control.
Thrombus Formation on Tissue Factor

TABLE 3. Relationship Between Coagulation Factors and Thrombus Formation on Tissue Factor

<table>
<thead>
<tr>
<th></th>
<th>Flunitdione</th>
<th>Flunitdione + Aspirin</th>
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<tr>
<td></td>
<td>Platelet Deposition</td>
<td>Fibrin Deposition</td>
</tr>
<tr>
<td>INR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.51</td>
<td>-0.53</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Factor II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>P</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Factor VII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.41</td>
<td>0.34</td>
</tr>
<tr>
<td>P</td>
<td>0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>Factor X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.54</td>
<td>0.54</td>
</tr>
<tr>
<td>P</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Factor IX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>P</td>
<td>0.08</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Flunitdione or flunitdione+aspirin was given to 10 healthy volunteers. Blood for measurement of prothrombin times, INR, and % activity of plasma coagulation factors was collected just before blood donation for experimental thrombogenesis. TF-coated coverslips were exposed for 3 minutes to native blood at a shear rate of 2600 s⁻¹. Platelet and fibrin deposition were determined by immunoenzymatic measurements.

3). Due to interrelationships between INR and the plasma levels of factors II, VII, IX, and X, we performed a multiple regression analysis to analyze the independence of the association. Using this test, we found that the antithrombotic effect of flunitdione was more specifically correlated to plasma levels of factor X (β=0.60, P<0.001 for platelet reduction and β=0.57, P<0.001 for fibrin reduction).

Effect of Treatment on Collagen-Induced Thrombus Formation

Thrombi that formed on collagen-coated coverslips in controls were rich in platelets and poor in fibrin (Figure 3). Platelet deposition was not reduced in subjects treated by flunitdione. In contrast, it was reduced by 48% in those treated with flunitdione plus aspirin, regardless of the level of anticoagulation (P<0.05 versus control). Similarly, fibrin deposition was not prevented by flunitdione alone, whereas it was decreased by 60% when flunitdione was combined with aspirin (P<0.05 compared with flunitdione alone). Probably due to large interindividual variations in fibrin deposition within control experiments (range 0.08 to 8.34 μg/cm²), the effect of an aspirin adjunct on fibrin deposition was not statistically significant compared with controls. The antithrombotic effect was correlated neither to INR nor to plasma levels of factors II, VII, IX, or X (P>0.5).

 Whereas platelet adhesion to collagen, as measured by morphometry, was not altered by flunitdione, there was a slight but significant increase of platelet adhesion in volunteers treated with aspirin plus flunitdione (13% enhancement compared with flunitdione INR 2.1 to 3.0, P<0.05; Table 2).

Collagen-dependent thrombus formation resulted in a lower activation of platelets and coagulation than TF-dependent thrombus formation: plasma levels of βTG and T-AT were respectively 2 and 8 times lower with collagen than with TF (Figures 2 and 4). βTG release was not prevented by flunitdione alone but was prevented by 55% when flunitdione was combined with aspirin (P<0.01 versus control and flunitdione alone). Neither treatment prevented T-AT formation.

Discussion

The place of vitamin K antagonists among the different therapeutic strategies for preventing arterial thrombus formation and the clinical benefit of adding aspirin to oral anticoagulants in this setting are still debated. In the present study, we demonstrated (1) that flunitdione is an effective agent for preventing experimental arterial thrombosis in humans, but that its efficacy is related to the plasma anticoagulation level and is dependent on the thrombogenic trigger, and (2) that combined aspirin plus flunitdione therapy is effective regardless of the thrombogenic trigger, which indicates that the antithrombotic potency of combined therapy is higher than that of flunitdione alone.

Flunitdione reduced fibrin and platelet deposition on TF in a dose-dependent manner (Figure 1). This finding is not surprising, because thrombin generation plays a key role in the formation of mixed fibrin- and platelet-rich thrombi on TF. However, flunitdione appears particularly effective and much more potent than unfractionated heparin. Because platelets are largely recruited into arterial thrombi by...
subject treated with fluindione plus aspirin, whereas it was deposition was therapy was effective in all subjects: platelet and fibrin of fluindione. First, conventional fluindione plus aspirin suggest that aspirin does enhance the antithrombotic efficacy one plus aspirin in a dose-response manner comparable to levels (INR 2.1 to 3.0). Similarly, clinical studies3–5 have platelet thrombus formation at conventional anticoagulation (Table 3). Fluindione was most effective in preventing the generation of thrombin and thus the amount of thrombus–vitamin K– dependent coagulation factors, fluindione reduces we presume that by decreasing the functional levels of the thrombus-bound thrombin rather than soluble thrombin,31–33 we presume that by decreasing the functional levels of the vitamin K–dependent coagulation factors, fluindione reduces the generation of thrombin and thus the amount of thrombuss-bound thrombin.

The antithrombotic effect of fluindione reflected the plasma level of anticoagulation, as expressed by the INR (Table 3). Fluindione was most effective in preventing platelet thrombus formation at conventional anticoagulation levels (INR 2.1 to 3.0). Similarly, clinical studies1–5 have shown that oral anticoagulants at high levels of anticoagulation were effective in secondary prevention of myocardial infarction. However, in the present study, fluindione at low anticoagulation levels (INR 1.5 to 2.0) also was effective in preventing TF-induced thrombus formation (Figure 1). This level of anticoagulation is reported to be effective in primary prevention of ischemic heart disease in men.6

TF-induced thrombus formation was inhibited by fluindione plus aspirin in a dose-response manner comparable to that of fluindione alone (Figure 1). Nevertheless, some data suggest that aspirin does enhance the antithrombotic efficacy of fluindione. First, conventional fluindione plus aspirin therapy was effective in all subjects: platelet and fibrin deposition was <1.3×10^7/cm^2 and <0.6 μg/cm^2 in each subject treated with fluindione plus aspirin, whereas it was not negligible in 3 subjects treated with fluindione alone, ie, ≥2×10^7/cm^2 and >1.7 μg/cm^2, respectively. In addition, the morphometric analysis indicated that by inhibiting fibrin deposition, fluindione plus aspirin reduced the percent of platelets adherent to fibrin meshes significantly more than fluindione alone (P<0.05; Table 2).

Fluindione exerted no antithrombotic effect on collagen-coated surfaces (Figure 3). Thus, the present data confirm that thrombin is not involved in the mediation of collagen-triggered initial thrombus formation at a wall shear rate of 2600 s^−1.17,24,29 However, because thrombin plays a major role in thrombus growth and stabilization,28,34 the late antithrombotic effects of vitamin K antagonists on collagen surfaces remain to be determined.

On collagen, the combination of aspirin and fluindione, regardless of the INR, resulted in a significant decrease in platelet and fibrin deposition (Figure 3). Because fluindione alone had no antithrombotic effect on this surface and because the effect of fluindione plus aspirin was comparable with low and conventional levels of anticoagulation, the antithrombotic efficacy of fluindione plus aspirin on collagen may reflect the effect of aspirin alone. Thus, in a previous study,26 platelet and fibrin deposition on collagen were prevented by aspirin in a comparable manner.

Platelet adhesion on collagen was increased in subjects treated with fluindione plus aspirin compared with subjects not given aspirin (Table 2). This also has been found in previous studies using comparable perfusion models.21–23,26 Indeed, when platelet consumption by growing thrombi is decreased by agents that interrupt platelet-platelet interactions, such as aspirin, there is concomitantly an increase in the platelet concentration in the blood layers streaming adjacent to the collagen surface, which results in more platelets available to adhere to collagen.

Overall, the antithrombotic effect of fluindione plus aspirin was greater than that produced by either drug used alone, because both TF- and collagen-triggered thrombus formation was prevented when these drugs were combined. The 2 agents exerted independent effects, because fluindione was principally responsible for the antithrombotic effect on TF, whereas aspirin was effective on collagen. These results are in agreement with clinical studies9 in which the addition of aspirin to oral anticoagulants in patients with mechanical heart prostheses was more effective in preventing systemic embolism than oral anticoagulants alone.

The antithrombotic effect of fluindione was correlated to plasma levels of factors II and X but not to plasma levels of factors VII and IX (Table 3). In addition, the antithrombotic effect was more correlated to plasma levels of factor X than to those of factor II. In rabbits, reduction of prothrombin and possibly of factor X was also more important than reduction of factor VII and IX for the anticoagulant effect of vitamin K antagonists.35 Thus, these results confirm the importance of the reduction of factors X and II for the antithrombotic effect of vitamin K antagonists, but it is possible that the respective roles of factors II and X depend on the type of thrombosis.

Finally, it has been reported that high doses of aspirin (>1500 mg/d) augment the anticoagulant effect of vitamin K antagonist.36 We did not observe such results with a lower dosage of aspirin (325 mg/d), because the daily dose of fluindione required to reach the target INR (Table 1) or the
time period needed to reach a stable level of anticoagulation (Table 1) was comparable even when fluindione and aspirin were combined.

References


Antithrombotic Efficacy of the Vitamin K Antagonist Fluindione in a Human Ex Vivo Model of Arterial Thrombosis: Effect of Anticoagulation Level and Combination Therapy With Aspirin

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