Dipyridamole Stimulates Urokinase Production and Suppresses Procoagulant Activity of Rabbit Alveolar Macrophages: A Possible Mechanism of Antithrombotic Action

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Dipyridamole, an inhibitor of platelet aggregation, has been shown to have beneficial effects in disorders characterized by extravascular fibrin deposition. Mononuclear phagocytes are present in extravascular sites and are capable of expressing both plasminogen activator and procoagulant activities, which suggests these cells play a central role in extravascular fibrin turnover. We therefore sought to determine whether dipyridamole affects the expression of plasminogen activator and procoagulant activities by rabbit alveolar macrophages cultured in vitro. We found that dipyridamole (10 to 100 μmol/L) caused increases in both cell-associated and released plasminogen activator activity, which reached levels of 240% (P < .05) and 543% (P < .01) of controls, respectively. In contrast, dipyridamole decreased the cell-associated procoagulant activity of alveolar macrophages to as little as 21.3% of controls (P < .01). Similar effects were seen in cells cotreated with lymphokines. The procoagulant activity expressed by these cells functioned as a tissue thromboplastin. The plasminogen activator of control and treated cells was a urokinase as determined by molecular weight characteristics (50 kilodaltons) and by antibody neutralization profiles using polyclonal antibodies against human urokinase and tissue plasminogen activator. These effects of dipyridamole could not be duplicated by structurally dissimilar agents sharing some of the pharmacological actions of dipyridamole; however, two pyrimidopyrimidine compounds structurally similar to dipyridamole effectively mimicked the effects on both procoagulant and plasminogen activator activities. We conclude that dipyridamole may have antithrombotic effects by directly modulating the role of mononuclear phagocytes in fibrin turnover. Thus, dipyridamole may be useful in situations where extravascular fibrin deposition is important to the pathogenesis of tissue injury and repair.

DIPYRIDAMOLE is a pyrimidopyrimidine compound widely used in treating thrombotic disease because of its actions as a platelet antiaggregant and vasodilator. In addition, dipyridamole has been used successfully either singly or in combined therapy for glomerulonephritis and in animal models of glomerulonephritis and disseminated intravascular coagulation. These diseases are characterized histologically by extravascular fibrin deposition as well as intravascular platelet-fibrin thrombi. However, it is not clear how dipyridamole may also have its antithrombotic effects in the extravascular space where platelet aggregation and altered blood flow may not be the prime determinants of fibrin deposition.

Mononuclear phagocytes express procoagulant activity mainly by producing a tissue thromboplastin that activates the extrinsic coagulation pathway. These cells may also contribute to fibrinolysis by producing a plasminogen activator or plasminogen-independent enzymes. We have previously demonstrated that rabbit alveolar macrophages produce both tissue thromboplastin and plasminogen activator activities. Since mononuclear phagocytes are often abundant at sites of inflammation, they may be important cellular mediators of tissue deposition and clearance of fibrin. Therefore, in this study we sought to determine whether dipyridamole may directly affect macrophage expression of procoagulant or plasminogen activator activities. Such an effect would offer a potential explanation for the modulation by dipyridamole of extravascular fibrin turnover in inflammatory foci.

MATERIALS AND METHODS

Animals. Normal male New Zealand white rabbits (1.5 to 2 kg) were given routine care in the Unit for Laboratory Animal Medicine, University of Michigan Medical Center.

Bronchoalveolar lavage. Rabbits were sacrificed by intravenous injection of pentobarbital and exsanguinated prior to lavage. The trachea was cannulated, and the lungs were lavaged with 35-mL saline at 37 °C (PBS; Gibco, Grand Island, NY) until a return of 300 mL was obtained. Cells were collected by centrifugation and resuspended in 110 mmol/L Tris buffer in 100 mmol/L NaCl (pH 7.0) for two minutes to lyse contaminating erythrocytes. The cells were then washed three times in serum-free culture medium consisting of RPMI 1640 (Gibco) containing penicillin (100 U/mL), streptomycin (100 μg/mL), gentamicin (100 μg/mL), polymyxin B (100 U/mL), and lactalbumin hydrolysate (0.2%; Gibco) and counted with a hemocytometer.

Preparation of additives. Dipyridamole (Sigma Chemical Co., St Louis) and related additives were freshly dissolved in 95% ethyl alcohol to 10 mmol/L and further diluted in culture medium. The final concentration of ethyl alcohol in cell cultures did not exceed 1% and was found to alter neither cell viability nor expression of procoagulant and plasminogen activator activities (data not shown).

Lymphocyte-conditioned medium (LCM) was used as a crude source of lymphokines. Lymphocyte suspensions were prepared from paraaortic lymph nodes as previously described. The cell suspensions (~95% lymphocytes) were incubated for 48 hours at 5 x 10⁶ cells/mL in medium containing concanavalin A bound to Sepharose 4B beads (5 μg/mL. ConA. Pharmacia Fine Chemicals, Piscataway, NJ). The LCM was then centrifuged to remove cells and ConA-
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Sepharose, sterilized by filtration, and stored at −70 °C. Preliminary data indicated that a 1:8 dilution of LCM was optimal, and one such preparation was used in these experiments.

**Cell culture.** One million cells were dispensed in 1 mL of culture medium and incubated in duplicate in sterile 12 × 75-mm polypropylene tubes for 24 hours at 37 °C in 5% CO2-enriched air. Dipyridamole and other additives were included in the culture medium for the entire incubation period as indicated. Following incubation, the cells were collected by centrifugation and resuspended in fresh medium. Cell suspensions and incubation media were stored at −20 °C prior to assay.

**Procoagulant assay.** The cells were lysed by two cycles of freeze-thawing followed by sonication. Preliminary experiments showed that the majority of the procoagulant activity remained cell associated in vitro because only minimal activity was found in the culture supernatants. None of the interventions tested caused a significant release of procoagulant activity into the incubation medium, thus indicating that modulation of procoagulant activity was adequately reflected by changes in the lysate activity. Accordingly, only cell lysates were routinely measured for procoagulant activity.

Procoagulant activity was assayed using a one-stage coagulation assay.14 One hundred microliters of test material was mixed with an equal volume of 25 mmol/L CaCl2 at 37 °C. Citrated normal rabbit plasma (100 μL) was then added, and the time elapsed until fibrin formation was measured with an automated coagulation timer (BBL Fibrosystem, Cockeysville, MD). Coagulation times of duplicate assays were reproducible within 12% over the range of activities encountered in this study. Standard curves were derived from serial dilutions of macrophage lysate by using a log-log plot of lysate concentration and the resultant coagulation time.14 Like identical assays using rabbit brain tissue thromboplastin (Sigma), these standards were linear for coagulation times ranging approximately from 20 to 260 seconds. Coagulation times could then be converted to arbitrary units of procoagulant activity to calculate the percent change from baseline activity induced by the various interventions.

To determine the mechanism of action of the procoagulant, samples were tested in coagulation assays using plasmas congenitally deficient in factors VIII, VII, or X (George King Bio-Medical, Overland Park, KS). In addition, the samples were pretreated with phospholipase C (Sigma), 0.05 μg/mL for 30 minutes at 37 °C because inactivation of procoagulant activity by this enzyme is indicative of tissue thromboplastin activity.11

**Plasminogen activator assay.** Incubation media and cell lysates were assayed for plasminogen activator activity using a modification of the 125I-fibrin plate assay of Unkeless et al.15 Fibrin plates were prepared by adding to 16-mm plastic culture wells 60 μg of purified human fibrinogen (KABI, Stockholm, Sweden) containing 2 x 10^6 cpm 125I-labeled fibrinogen (Amersham Corp, Arlington Heights, IL) and drying the plates at 37 °C. The wells were then incubated with 5% acid-treated fetal bovine serum (GIBCO) to convert the fibrinogen to fibrin and then washed thoroughly with 0.1 mol/L Tris buffer (pH 8.1) prior to use. Plasminogen was prepared from outdated human plasma by lysine-Sepharose affinity chromatography (Pharmacia) according to the method of Deutsch and Mertz18 and quantified by protein content.19 To inactivate contaminating plasmin, the plasminogen preparation was incubated with phenylmethylsulfonyl fluoride (1 mmol/L) for 16 hours at 4 °C followed by extensive dialysis. The plasminogen concentration that produced the maximal fibrinolysis with a standard sample of plasminogen activator was used in all assays (2 to 6 μg/mL).

To assay for plasminogen activator activity, test materials were added in duplicate with either plasminogen or buffer to 125I-fibrin-coated wells in a final volume of 1 mL. Following incubation for four hours at 37 °C, an aliquot of incubation medium was then removed and 125I activity measured with a gamma counter. Control wells containing either plasminogen or buffer alone were also included in each assay. Plasminogen activator activity was calculated as the difference between 125I release in wells containing test material with plasminogen and the largest control value. The net 125I release was expressed as the percentage of maximal release achieved by trying the wells. The resultant percent lysis was translated into milli-Plough units (mPU) by using a standard curve generated from human urokinase (Calbiochem, La Jolla, CA). Plasminogen-independent fibrinolysis was negligible in all samples and accounted for less than 5% of the fibrinolysis seen in the presence of plasminogen.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).** Culture supernatants from alveolar macrophages (10^6/mL) incubated for 24 hours in standard medium or medium containing dipyridamole (100 μmol/L) were concentrated 40-fold by pressure dialysis (Amicon Ultrafiltration System, Diaflo P10 membrane, Amicon Corp, Lexington, MA). Concentrated supernatants and standard samples of human urokinase and tissue plasminogen activator (tPA; American Diagnostica, Greenwich, CT) were treated with 10% SDS/8 mol/L urea. The samples were then subjected to electrophoresis at 20 mA on 60 × 100 × 0.4-mm gels of 11% polyacrylamide with 10% SDS in a Laemmli buffer system under nonreducing conditions.20

**Fibrin-agar zymography.** Fibrin-agar indicator plates were created by pouring agarose (1.25%) containing fibrinogen (2 mg/mL), thrombin (Sigma; 0.06 National Institutes of Health U/mL), and plasminogen (5 μg/mL) onto glass slides.21 Following completion of electrophoresis, the polyacrylamide gels were washed sequentially in Triton X-100 (2.5%) and water in preparation for assay on the fibrin-agar plates. The gels were then layered over the indicator plates and incubated at 37 °C for six to eight hours. The indicator plates were then stained with amido black, and the plasminogen activator activity was located by identifying the zones of lysis on the indicator plates.

**Antibody neutralization.** The plasminogen activator activities of control and dipyridamole-treated culture supernatants were characterized by neutralization profiles with antibodies against human urokinase and tPA. Immunoglobulin from antihuman urokinase goat serum (generously provided by G. Murano, PhD; Department of Biologicals, Food and Drug Administration, Bethesda, MD) was purified by diethyl amineethyl chromatography and ammonium sulfate precipitation.22 Goat antihuman tPA immunoglobulin prepared in like fashion was purchased from American Diagnostica. Antibody concentrations were determined by the protein content.19 Test samples and the two plasminogen activator standards adjusted to equivalent activity were preincubated with a range of concentrations of antibody prior to the measurement of plasminogen activator activity. In these experiments, the plasminogen activator activity was measured using the two-stage spectrophotometric method of Coleman and Green.23 The percent inhibition by antibody was calculated using standard curves for plasminogen activator activity generated for each sample.

**Statistical analysis.** For all dose-response curves, multiple comparisons with control groups were performed using Dunnett’s test applied to a one-way analysis of variance.24 Single comparisons were made using the unpaired two-tailed Student’s t test.24

**RESULTS**

**Cell culture.** The cell preparations used in these experiments were consistently more than 95% alveolar macrophages as described previously.15 In all cases, cell viability was greater than 95% prior to incubation as determined by trypan blue exclusion. Following incubation for 24 hours in
medium alone, the viability was 90% ± 7% (mean ± SD). The viability was not significantly reduced by dipyridamole in concentrations ranging from 1 μmol/L (90.1% ± 9.4%) to 50 μmol/L (80% ± 15%).

The procoagulant activity of cells cultured in medium alone remained low over 24 hours (108.6 ± 15 seconds). Since augmented expression of procoagulant activity by these cells is a consistent response to low concentrations of endotoxin, this served as an internal control to indicate that the cells were not being subjected to significant background stimulation by endotoxin in the culture system.

Effects of dipyridamole on alveolar macrophage plasminogen activator activity. In control cultures incubated in standard medium alone, the plasminogen activator activity of cell lysates was 448 ± 101 mPU/10^6 cells; in addition, 462 ± 11 mPU/mL was present in the incubation medium (mean ± SEM). Dipyridamole stimulated marked increases in the plasminogen activator activity released into the medium, which reached a maximum of 543% of control cultures (P < .01; Fig 1). There was also a significant increase in the activity of cell lysates, which reached 240% of the controls (P < .05), thus indicating that dipyridamole augmented the total expression of plasminogen activator rather than simply stimulating the release of intracellular stores of the enzyme into the culture media. Plasminogen activator activity of maximally stimulated cells (50 μmol/L dipyridamole) was 1,132 ± 322 mPU/10^6 cells in lysates and 1,619 ± 774 mPU/mL in media, both significantly increased from control values (P < .05).

Dipyridamole had no direct effect on plasminogen activator activity when mixed with cell lysates, thereby indicating that the drug did not activate an existing proenzyme. Finally, stimulation of plasminogen activator production was completely blocked by cycloheximide (5 μg/mL), which indicated a requirement for protein synthesis (not shown).

Characterization of unstimulated and dipyridamole-stimulated plasminogen activator. Plasminogen activator activities of incubation media from control and dipyridamole-treated alveolar macrophages were significantly inhibited by anti-human urokinase antibody (Fig 2A). A human urokinase standard with equivalent activity was similarly inhibited by this antibody, but there was little cross-reactivity with human tPA. The anti-tPA antibody had only a slight neutralizing effect on the control media and none on media from dipyridamole-treated cells (Fig 2B). This was comparable to the effect of antibody on urokinase standard, whereas tPA was completely neutralized. These results indicate that the plasminogen activator of normal and dipyridamole-treated alveolar macrophages is a urokinase rather than a tPA.

SDS-PAGE analysis of incubation media from both control and dipyridamole-treated cells revealed a single band of plasminogen activator activity with a molecular weight of approximately 50 kilodaltons (kD) (Fig 3). A human urokinase standard demonstrated a major band of activity of 53 kD and a minor band of 32 kD, whereas human tPA produced a single band of 68 kD. Together with the antibody neutralization experiments, these results indicate that dipyridamole caused increased production of a single form of urokinase rather than stimulating production of a second protease with plasminogen activator activity.

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Fig 3. Molecular weight determination of rabbit alveolar macrophages plasminogen activator. Shown is the fibrin-agar indicator plate developed from an SDS-PAGE (see Materials and Methods). Human urokinase (lane 1) demonstrated a major band at 53 kD and a minor band at 32 kD, whereas human tPA produced a single band at 68 kD (lane 2). Incubation media from both dipyridamole-treated (lane 3) and untreated (lane 4) macrophages produced a single band of activity at 50 kD, virtually identical to the major band of human urokinase.

From control cultures were somewhat variable, but dipyridamole consistently and significantly decreased the expression of procoagulant activity in parallel cultures to as little as 21.3% of control values (P < .01; Fig 4A). The corresponding prolongation in coagulation time was also significant, increasing from 108.6 ± 15 seconds in controls to 190.1 ± 58.7 seconds in cells treated with 50 μmol/L dipyridamole (P < .05). To determine whether dipyridamole also suppressed the augmentation of procoagulant activity in response to an immunostimulant, alveolar macrophages were cocultured with dipyridamole and LCM. In these experiments, dipyridamole (10 μmol/L and 100 μmol/L) also decreased the procoagulant activity by as much as 83.3% (Fig 4B). The coagulation times were prolonged from 66.8 ± 10.7 seconds (LCM alone) to 96.5 ± 15.7 seconds (10 μmol/L, P < .01) and 171.3 ± 23.2 seconds (100 μmol/L, P < .01). Additional experiments verified that dipyridamole had similar suppressive effects on the procoagulant activity expressed by suspensions of intact cells (not shown).

Dipyridamole had no direct effect on the coagulation assay. Second, the loss of procoagulant activity in dipyridamole-treated cells was not affected by incorporating excess phospholipid (rabbit brain cephalin, Sigma) in the assay system, which suggests that the drug did not simply alter the phospholipid content of the lysates. Finally, in mixing experiments the addition of lysates of dipyridamole-treated cells lacking detectable procoagulant activity did not inhibit the activity of lysates of control cells, which indicates that dipyridamole did not stimulate the expression of an inhibitor of procoagulant activity. The procoagulant activity of both untreated and dipyridamole-treated cell lysates functioned as tissue thromboplastin. This was demonstrated by a loss of procoagulant function in plasmas deficient in factors VII or X but not VIII (not shown). In addition, procoagulant activity was inactivated by pretreatment by phospholipase C, which inactivates tissue thromboplastin but not other coagulation factors.

Mechanism of action of dipyridamole. Experiments were performed to determine the mechanisms by which dipyridamole modulates expression of macrophage procoagulant and plasminogen activator activities. The effects of dipyridamole were (1) compared with those of structurally unrelated compounds sharing at least one of the known pharmacological actions of dipyridamole, (2) examined in the presence of agents that antagonize specific actions of dipyridamole, and (3) compared with those of structurally related pyrimidopyrimidines.

Dipyridamole is known to alter production and cellular responses to various arachidonic acid metabolites. To deter-
Dipyridamole is known to inhibit the transport of adenosine across plasma membranes, which leads to extracellular accumulation of adenosine and increased stimulation of plasma membrane adenosine receptors. To determine whether dipyridamole modulates procoagulant and plasminogen activator activities by blocking adenosine transport, we compared cells treated with dipyridamole with those treated with nitrobenzylthioinosine (NBTI, 0.1 to 100 μmol/L), another inhibitor of adenosine transport. NBTI did not mimic the effects of dipyridamole (Fig 5B). Second, we compared cells treated with dipyridamole with those treated with nitrobenzylthioinosine (NBTI, 0.1 to 100 μmol/L), another inhibitor of adenosine transport. NBTI did not mimic the effects of dipyridamole, but caused only moderate suppression of procoagulant activity, and had no effect on the plasminogen activator activity (Fig 5B). These results indicate that the action of dipyridamole is not based on the inhibition of adenosine transport across cell membranes.

Dipyridamole is known to inhibit cyclic adenosine monophosphate (AMP)-phosphodiesterase. To determine whether this action pertains to its effects on procoagulant and plasminogen activator expression, we compared the effects of dipyridamole with those of other phosphodiesterase inhibitors, papaverine (10 μmol/L) and methylisobutylxanthine (MIX, 100 μmol/L). In contrast to the effects of dipyridamole, papaverine significantly inhibited both plasminogen activator and procoagulant activities by 56.5% (P < 0.01) and 45% (P < 0.05), respectively (Fig 5C). MIX inhibited plasminogen activator activity by 69% (P < 0.01) but had no significant effect on procoagulant activity. These results indicate that inhibition of cyclic AMP phosphodiesterase cannot fully explain the actions of dipyridamole. The mechanism of action of these phosphodiesterase inhibitors is likewise unclear. We have recently shown that dibutyl cyclic AMP suppresses plasminogen activator secretion but not cell-associated procoagulant activity, thus indicating that the effects of papaverine and MIX cannot be explained solely by a global increase in intracellular levels of cyclic AMP (Hasday and Sitrin, manuscript submitted).

Finally, experiments were performed to investigate whether the effects of dipyridamole were shared by other phosphodiesterase inhibitors. Alveolar macrophages were treated with RXRA-85 (4-[1-oxidothiomorpholino]-8-[2-phenyl-ethylthyl]-2-piperizino-pyrardo/5, 4-d/pyrimidine) and RE-102 (3,6-dimorpholino-8-[bis-(2-hydroxypropyl)-amino-2-phenyl-pyrimido-(5,4-B)pyrinez), two structural analogues of dipyridamole kindly provided by the Boehringer-Ingelheim Co (FR Germany). The effects of optimal concentrations of these compounds on the expression of procoagulant and plasminogen activator activities are shown in Fig 6. Both RXRA-85 (10 μmol/L) and RE-102 (100 μmol/L) significantly inhibited expression of procoagulant activity (69.7% and 92.3% respectively, P < 0.01). In addition, the release of plasminogen activator into culture media was markedly stimulated (325% and 486% respectively, P < 0.01), effectively duplicating the effects of dipyridamole.
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The close association between inflammatory reactions and activation of the coagulation and fibrinolytic systems is well recognized. The clinical manifestations of these interrelationships include disseminated intravascular coagulation and localized formation of intravascular fibrin-platelet thrombi. In addition, fibrin deposition outside the vascular space is prominent in many inflammatory states such as cutaneous hypersensitivity, glomerulonephritis, and acute lung injury. Various methods of anticoagulation have suppressive effects on cutaneous hypersensitivity and glomerulonephritis, which suggests that products of coagulation may intensify inflammation. In vitro experiments have demonstrated that fibrinogen-related products are leukocyte chemotaxins, impair macrophage mobility, and induce microvascular permeability.

Although the factors controlling extravascular fibrin turnover are not well elucidated, there is considerable evidence to suggest that mononuclear phagocytes are central to this process. These cells can directly promote coagulation by elaborating tissue thromboplastin and other coagulation factors as well as mediating fibrinolysis by producing plasminogen activator or fibrinolytic enzymes. Both tissue thromboplastin and plasminogen activator activities can be stimulated in vitro by many stimuli relevant to inflammation including lymphokines and bacterial lipopolysaccharides. Recent studies have suggested that macrophage-derived procoagulant activity contributes to local fibrin deposition in experimental glomerulonephritis, thereby supporting the potential importance of these activities in vivo. Further, Chapman et al have demonstrated abnormal expression of procoagulant and plasminogen activator activities by alveolar macrophages from some patients with interstitial lung disease. Currently, there are few options for pharmacologic manipulation of the effect of macrophages on fibrin deposition and clearance. Glucocorticosteroids, indomethacin, Coumadin, and prostaglandin E modify expression of procoagulant or plasminogen activator activities in vitro, but no other agents potentially applicable to in vivo use have been identified.

Several studies have demonstrated beneficial effects of dipyridamole in glomerulonephritis and attributed these to its actions as an inhibitor of platelet function. Since the crescentic fibrin deposition seen in an animal model appears to be caused by infiltrating macrophages, we sought to determine whether dipyridamole directly modulates the expression of procoagulant or fibrinolytic activities of mononuclear phagocytes. Using normal rabbit alveolar macrophages, we found that dipyridamole had profound effects on the expression of both activities in vitro because expression of tissue thromboplastin activity was suppressed by over 80% whereas the concurrent release of plasminogen activator was increased by more than fivefold. The suppression of tissue thromboplastin activity occurred by decreased cellular expression of this factor rather than by stimulating production of an inhibitory protein or by directly affecting the extrinsic coagulation pathway. Likewise, dipyridamole directly stimulated cellular production of plasminogen activator rather than causing the release of the enzyme from intracellular stores or by activating a preexisting proenzyme. The plasminogen activator was found to be a single protein with molecular weight and immunologic characteristics of urokinase. The mechanisms underlying these actions of dipyridamole remain undefined. We determined that structurally unrelated compounds sharing at least one of the well-recognized pharmacological actions of dipyridamole, namely cyclic AMP-phosphodiesterase inhibition, blockage of purine transport across plasma membranes, and alteration of arachidonic acid metabolism could not reproduce both these effects. However, two other structurally related pyrimidopyrimidines were roughly equipotent in their effects on both tissue thromboplastin and urokinase activities.

Further studies are certainly necessary to determine whether dipyridamole has comparable effects on mononuclear phagocytes in vivo. However, the potential biologic relevance of the findings reported here is supported by the observation that dipyridamole affected both activities in vitro at concentrations ranging from 10 to 25 μmol/L. Plasma concentrations in excess of 10 μmol/L can be achieved in some human subjects with the peak effect of a single 75-mg dose, and considerably higher doses have been given safely.

The results of this study suggest several important areas for future investigations. First, it will be necessary to explore the possibility that dipyridamole and related compounds can be therapeutically informative in inflammatory disorders by preventing fibrin from accumulating in tissues. These studies should also better define the importance of macrophage-directed fibrin turnover in the pathogenesis of inflammation and healing. One potential advantage to this approach is that fibrin deposition may be selectively suppressed in injured tissue where macrophages have accumulated without inducing a severe generalized hemostatic defect. Second, the possibility is raised that dipyridamole may have other striking effects on macrophage functions beyond the two activi-
ties reported here, thereby suggesting that the pyrimidopyrimidines represent a new class of pharmacological agents for modulating mononuclear phagocyte function in inflammatory diseases in vivo. Further supporting this hypothesis are limited studies demonstrating that dipyridamole inhibits lymphoproliferation and stimulates interferon secretion by leukocytes in vitro. Such investigations may also provide important new information on the mechanisms underlying the pharmacological actions of pyrimidopyrimidines as well as the expression of macrophage effector functions. Finally, these actions of dipyridamole may have important implications for investigations of atherosclerotic diseases. Prior studies have demonstrated that macrophages infiltrate atheromas and release factors that may contribute to their progression. Moreover, dipyridamole has inhibited plaque formation in experimental atherosclerosis. Given the current evidence for the role of thrombus formation in coronary artery occlusion and the widespread interest in thrombolytic and anticoagulant therapies for management of myocardial ischemia, the possibility for pharmacologically suppressing some of the thrombogenic activity in atheromas while concurrently increasing local fibrinolysis certainly merits further investigation.

In summary, we have demonstrated that dipyridamole causes alveolar macrophages to decrease the expression of tissue thromboplastin activity while concurrently stimulating the production and release of urokinase. These combined actions suggest that dipyridamole may effectively prevent fibrin accumulation at sites of inflammation through its direct effects of mononuclear phagocytes.

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