3-Deazaadenosine Inhibits Thrombin-stimulated Platelet-derived Growth Factor Production and Endothelial-Leukocyte Adhesion Molecule-1-mediated Monocytic Cell Adhesion in Human Aortic Endothelial Cells*

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Injury to the vascular endothelium and the subsequent inflammatory response are considered prerequisites for the development of atherosclerosis. Platelet-derived growth factor (PDGF) production by and monocytic cell adhesion to aortic endothelial cells (EC) may participate in this inflammatory process and therefore are two potential targets for control by anti-inflammatory agents. Our previous studies have demonstrated that monocyte adhesion and PDGF production are stimulated by thrombin in EC. Here, we provide evidence that treatment of EC with the anti-inflammatory agent 3-deazaadenosine (c3Ado) effectively abolished thrombin-stimulated PDGF production and monocyte adhesion. c3Ado had no significant effect on either basal monocyte adhesion or constitutive PDGF production. c3Ado was also effective in negating monocyte adhesion induced by other agonists, such as interleukin-1, phorbol 12-myristate 13-acetate (PMA), and lipopolysaccharide. Northern analysis demonstrated that c3Ado significantly reduced thrombin- and PMA-stimulated steady-state levels of PDGF-A chain, PDGF-B chain, and endothelial-leukocyte adhesion molecule-1 (ELAM-1) mRNAs. Nuclear run-on studies demonstrated that a marked transcriptional activation of these genes by thrombin and PMA was abrogated by c3Ado treatment. The transcriptional rate of the a-tubulin gene was unaffected by the drug. Antibody binding studies with an anti-ELAM-1 monoclonal antibody 7A9 revealed that thrombin-stimulated EC expression of ELAM-1 was abolished by c3Ado, indicating that suppression of ELAM-1 expression on EC surface may be a mechanism by which c3Ado interferes with monocyte adhesion. Experiments with the nucleoside transport inhibitor nitrobenzylthionine suggested that the transport of c3Ado into EC was required for its inhibitory activity. In addition, L-homocysteine thiolactone was found to potentiate the inhibitory activity of c3Ado, suggesting that the accumulation of intracellular c3Ado homocysteine may be the underlying mechanism by which c3Ado inhibits thrombin-induced EC function. Taken together, these results indicate that c3Ado may prove effective against vascular injury and inflammation through its ability to inhibit induction of both monocyte adhesion and PDGF production.

Inflammation of the vessel wall and the resultant adhesion of leukocytes to the endothelium are considered integral to both physiological and pathological processes such as wound healing, vasculitis, and atherogenesis. This inflammatory process is either the cause or the effect of the activation of endothelial cells (EC). Once activated, EC acquire the ability to perform a variety of new or altered cellular functions due to quantitative changes in certain gene products (for review, see Ref. 1). Several humoral agonists, such as lipopolysaccharide, cytokines, and thrombin are capable of activating EC, and as a result, change the net production of many bioactive substances.

The protease thrombin, besides being a key enzyme in the coagulation cascade, has been shown to stimulate several EC functions. Thrombin stimulates EC in vitro to enhance the production of prostacyclin (2, 3), platelet-activating factor (4, 5), endothelin (6), von Willebrand factor (7), plasminogen activator (8, 9) and its inhibitor (10), and platelet-derived growth factor (PDGF) (11-15). In addition, thrombin induces both monocyte and neutrophil adhesion to cultured EC (16, 17). Recent studies have demonstrated that the adhesion of leukocytes to activated EC is mediated through the expression of specific cell surface receptors (18-21). These inducible EC proteins appear to be necessary for the recruitment of leukocytes into the subendothelial space (22).

Unactivated EC express few binding sites either for monocytes or for neutrophils. When activated by specific agonists such as IL-1b, tumor necrosis factor-α, bacterial lipopolysaccharide, and thrombin, EC bind more monocytes and neutrophils due to the expression of specific cell surface proteins (16, 23-26). One such cell surface adhesion molecule is endothelial-leukocyte adhesion molecule-1 (ELAM-1) (18). Antibody binding studies have implicated this 115-kDa glycoprotein molecule as the adhesion molecule responsible for neutrophil adhesion (18). In addition, we have recently been able to demonstrate a role for ELAM-1 in monocyte adhesion using a specific monoclonal antibody (27). In view of the role

* The abbreviations used are: EC, endothelial cells; PDGF, platelet-derived growth factor; ELAM, endothelial-leukocyte adhesion molecule; IL, interleukin; c3Ado, 3-deazaadenosine; FBS, fetal bovine serum; PMA, phorbol 12-myristate 13-acetate.

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9376
of EC-leukocyte interactions in inflammation and the possible role of EC adhesion molecules in atherogenesis, monocyte adhesion to EC is a potential target for treatment with anti-inflammatory agents.

3-Deazaadenosine (c\(^3\)A) is a structural analog of adenosine that exhibits multiple biological effects in several experimental systems. In vitro, this drug inhibits monocyte and neutrophil chemotaxis (28-30), lymphocyte-mediated tumor cell lysis (31), macrophage phagocytosis and microfilament disorganization (32-34), histamine release by basophils (35), macrophage lysosomal secretion (36), and superoxide anion generation (37). In addition, c\(^3\)A has been shown to be immunosuppressive in mice (38). Recently, Jurgensen et al. (39) have demonstrated that c\(^3\)A can cause a parallel reduction in tumor necrosis factor-\(\alpha\)-induced neutrophil adhesion to human EC and a selective decrease in the steady-state levels of interleukin adhesion molecule-1 mRNA.

Despite the evidence in favor of the direct or indirect "anti-inflammatory" properties of c\(^3\)A and its use in ongoing clinical trials as a therapeutic agent for patients with rheumatoid arthritis (39), the molecular mechanisms underlying the diverse effects of the compound are not clearly understood. Inhibition of cellular methylation reactions by c\(^3\)A has been proposed as one of the possible mechanisms of its inhibitory action (40, 41). Treatment of cells with c\(^3\)A results in intracellular accumulation of two principal compounds, namely S-adenosylhomocysteine and S-3-deazaadenosylhomocysteine, both of which are known competitors of S-adenosylmethionine in methyltransferase reactions (42, 43). However, several investigators have shown that c\(^3\)A is capable of eliciting its biological responses independent of the methyltransferase pathway (30, 44-46).

In the present study, we have examined the effect of c\(^3\)A on two thrombin-stimulated EC functions. We present evidence that c\(^3\)A inhibits thrombin-induced monocyte adhesion and PDGF production by abolishing induced increases in the transcriptional rate of the genes for PDGF (A and B chains) and ELAM-1.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine \(\alpha\)-thrombin was purchased from U. S. Biochemical Corp. MCDB 106 was procured from Sigma. 3-Deazaadenosine (c\(^3\)A) was purchased from Southern Research Institute (Birmingham, AL); Na\(^{32}\)P and [\(^{3}H\)]leucine were purchased from ICN Biomedical Inc. (Irvine, CA). 1,10-Phenanthroline was procured from Amersham Corp. 3\(^2\)PUTP, [\(^{32}\)P]UTP, and sodium \([\alpha-\text{32P}]\)chromate were the products of Du Pont-New England Nuclear. All tissue culture plasticware was purchased from Costar (Cambridge, MA).

**Culture of Human Aortic EC**—Human aortic EC were isolated by collagenase digestion from infrarenal arteries, obtained from the surgical transplant unit of the Cleveland Clinic Foundation, by a modification of a published procedure (47). Briefly, aortic segments were opened longitudinally and rinsed in serum-free medium, and the exposed intimal surface was digested with collagenase (2 mg/ml in serum-free medium) for 15 min at 37 °C. The detached EC patches were gently collected with a rounded spatula or cotton swab and grown in primary culture on fibronectin-coated plates with MCDB 107 containing 15% FBS, 90 mg/ml heparin, and 150 mg/ml endothelial cell growth supplement derived from bovine hypothalamus and 4% CO\(_2\) at 37 °C in 5% CO\(_2\). The conditioned medium at 37 °C in 5% CO\(_2\), or the day of the experiment, the monolayers were washed once with serum-free medium and incubated with appropriate doses of the test reagents in medium in the presence or absence of thrombin for 4 h at 37 °C. The conditioned medium was then aspirated, the cells were washed once with phosphate-buffered saline (PBS), and the total RNA was extracted with guanidium isothiocyanate (4.2 m guanidinium isothiocyanate, 18 mM Särkoseyl, and 0.7% \(\beta\)-mercaptoethanol). RNA was obtained by isopycnic centrifugation over CsCl (55) and separated by electrophoresis on a formaldehyde denaturing gel. RNA was then transferred from the gel to a Nytran (Schleicher & Schuell) membrane by capillary transfer and hybridized with \([\alpha-\text{32P}]\)UTP-labeled cDNA probes for human PDGF-A chain (1.3-kilobase cDNA in PUC-18(amp))

**Assay for Monocyte Cell Adhesion to EC**—U937 cell adhesion to cultured human aortic EC was measured as previously described (60). Briefly, human aortic EC were plated into 24-well plates in MCDB 107 medium 48-96 h prior to the assay and grown to confluence (approximately 2 \times 10\(^5\) cells/well). On the day of the assay, U937 cells (5 \times 10\(^5\)/ml) were labeled for 90 min at 37 °C with 100 \(\mu\)Ci of \({\text{[14C]sodium chromate}}\) in culture medium (1 ml). The labeled cells were centrifuged by sedimentation, and 10\(^2\) viable U937 cells were added to each well of EC after removal of the incubation medium. The binding was performed at 4 °C for 1 h, the wells were washed, and the cells were lysed with 1% Triton X-100 prior to removing an aliquot for \(\gamma\)-radiation counting. The number of U937 cells bound per well was calculated from the initial specific radioactivity (c\(^3\)A/mC) of the labeled cells. All data points represent triplicate determinations, with S.E. less than 20%. Spontaneous release of \(\beta\)-CR from the monocyte cells during the assay incubation was less than 5% of the total count.

**Radioceptor Assay for PDGF**—Confluent human aortic EC in 12-well plates were incubated in MCDB 107 with 5% FBS for 6 h at 37 °C. The conditioned medium was then collected, and the PDGF concentration was measured by the ability of the unlabeled PDGF in the conditioned medium to compete with \(^{125}\)PDGF for receptor sites on cultured human foreskin fibroblasts (61). Homogeneous PDGF was purified from human platelets by a modification of the method of Raines and Ross (52) and used to standardize the assay system. PDGF was radiolabeled with Na\(^{32}\)P as described (53).

**Protein Synthesis**—Human aortic EC protein synthesis was determined by the incorporation of \([\text{H}]\)leucine into trichloroacetic acid-precipitable material, as we have previously described (54). Briefly, confluent human aortic EC were incubated for 5 h with \([\text{H}]\)leucine in the presence of the PDGF assay in the presence of 2 \(\mu\)g/ml \(\beta\)-mercaptoethanol. Amino acid was obtained by isopycnic centrifugation over CsCl (55) and separated by electrophoresis on a formaldehyde denaturing gel. RNA was then transferred from the gel to a nylon (Schleicher & Schuell) membrane by capillary transfer and hybridized with \([\alpha-\text{32P}]\)UTP-labeled cDNA probes for human PDGF-A chain (1.3-kilobase cDNA in PUC-18(amp)), PDGF-B chain (c-six 2.9-kilobase cDNA in pCDV1(amp)), ELAM-1 (1.85-kilobase cDNA in CDN(amp)), and chicken \(\alpha\)-tubulin (1.4-kilobase cDNA in pBR322(seti)). PDGF-A chain and ELAM-1 cDNAs were the generous gifts of Dr. C. Betsholtz of the University of Uppsala, Sweden and Dr. N. Newman of Otsuka America, Rockville, MD, respectively.

**Run-Off Transcription Assay**—Confluent human aortic EC (10-12 \(\times 10^6\) cells) were harvested by scraping in 2 ml of ice-cold Ca\(^2+\)-Mg\(^2+\)-free phosphate-buffered saline and washed once with PBS at 800 \(\times g\) for 5 min at 4 °C. The cells were then resuspended in ice-cold lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl\(_2\), and 0.5% Nonidet P-40) and disrupted using a 10-ml syringe with a 18-gauge needle. After incubating the suspension on ice for 5 min, the nuclei were collected by centrifugation as above, resuspended in 0.2 ml of 50 mM Tris, 5 mM MgCl\(_2\), 0.1 mM EDTA, and 40% glycerol buffer, pH 8.3. Nuclei were stored in liquid nitrogen until required. Run-off assays were performed using \([\alpha-\text{32P}]\)UTP as described previously (56). The labeled RNA was heated at 100 °C for 10 min, chilled on ice, and hybridized to slot-blotted target DNA on nylon filters in 5% formic acid.

**Antibody Binding to Endothelial Cells**—Human aortic EC were plated in 48-well plates and grown to confluence (48-72 h). At the time of the assay, the incubation medium was removed, the cells were washed twice with MCDB 107 media containing 1% bovine serum albumin, and intact antibody 7A9 (1 \(\mu\)g/ml) (a monoclonal antibody against ELAM-1 (20), generously provided by Dr. W. Newman) was applied in 150 \(\mu\)l of wash media and incubated at 4 °C for 1 h. After washing the wells thrice, biotin-conjugated, affinity-purified F(ab')\(_2\) fragment goat anti-mouse IgG + IgM (H + L) (Jackson Immuno Research Laboratories, PA) conjugated to 125\(\beta\)labeled streptavidin (57) was added (150 \(\mu\)l/well), and the plate was incubated at 4 °C for 30 min. The wells were then washed three times, 80 \(\mu\)l of a \(^{125}\)I-labeled streptavidin solution (1:10-1:20 dilution in wash media) was applied per well, and the wells were reincubated at 4 °C for 15 min. Subsequently, the wells
c³Ado Inhibits Thrombin-stimulated ELAM-1 and PDGF Expression

were washed four more times, the cells were lysed with 1% Triton X-100, and an aliquot was removed for radiolabel quantitation. A nonspecific isotype control protein MOPC21 (a mouse myeloma IgG1-κ-protein) was included as a negative control to ascertain the specificity of the 7A9 antibody.

RESULTS

Concurrent incubation of confluent human aortic EC with c³Ado inhibited thrombin-stimulated PDGF production in a concentration-dependent manner (Fig. 1). Half-maximal inhibition was achieved at 30 µM c³Ado and maximal inhibition at 100 µM. These levels are consistent with the observed effective inhibitory concentration of c³Ado against a variety of biological functions (30, 37, 39). Constitutive PDGF production by the EC was unaffected by c³Ado treatment. Total cellular, as well as secreted protein synthesis by the EC, were not influenced by concentrations of up to 200 µM c³Ado (Fig. 1).

Unstimulated, confluent human aortic EC exhibit very little binding of the human monocytic cell line U937 cells (50). However, monocytic cell adhesion was substantially increased when the human aortic EC were treated with such agonists as thrombin, IL-1, and PMA (Fig. 2). Among the agonists tested, PMA induced the greatest adhesion followed by IL-1 and thrombin. c³Ado inhibited U937 cell adhesion stimulated by all three agonists in a dose-dependent fashion. c³Ado by itself exerted little influence on the basal level of monocytic cell adhesion. In addition, the inhibitory effect of c³Ado is not attributable to the interaction of the drug with U937 cells for two reasons. First, human aortic EC monolayers were washed prior to the addition of c³Ado into U937 cells. Second, addition of c³Ado to human aortic EC at the time of addition of 51Cr-labeled U937 cells did not affect either basal or stimulated monocytic cell adhesion (data not shown).

A possible mechanism by which c³Ado may exert its inhibitory activity is through the inhibition of S-adenosylhomocysteine hydrolase (40, 41). Inhibition of this enzyme results in an intracellular accumulation of S-adenosylhomocysteine, which is a potent inhibitor of transmethylation reactions requiring S-adenosylmethionine. Exogenous addition of homocysteine thiolactone, which results in S-adenosylhomocysteine accumulation has been shown to potentiate c³Ado activity in cell culture systems (39, 45, 57). When human aortic EC were coincubated with homocysteine thiolactone (100 µM) and c³Ado, the c³Ado inhibition of thrombin-induced monocyte adhesion and PDGF production was significantly potentiated. In the presence of homocysteine thiolactone and c³Ado, the half-maximal inhibitory concentration (IC₅₀) for thrombin-stimulated PDGF production in this experiment was 15 µM, as compared with 50 µM for c³Ado alone. Similarly, the IC₅₀ for thrombin-stimulated U937 cell adhesion was shifted in the presence of homocysteine thiolactone and c³Ado to 30 µM, as compared with 120 µM in the presence of c³Ado alone.

Prus and Zimmerman (58) have demonstrated in an erythrocyte model that c³Ado is transported into cells through an adenosine transporter. To determine whether c³Ado exerts its effects on thrombin-stimulated PDGF production and monocyte adhesion by acting intracellularly versus extracellularly, we used the adenosine transport inhibitor nitrobenzylthioinosine (39, 58) (Fig. 3). When human aortic EC were concurrently treated with c³Ado and nitrobenzylthioinosine, the inhibitory effect of c³Ado on thrombin-stimulated monocyte adhesion and PDGF production was prevented. However, nitrobenzylthioinosine was unable to inhibit the actions of c³Ado if it was added 60-90 min after the addition of c³Ado (data not shown). These results suggest that c³Ado exerts its cellular effects following transport into human aortic EC through an adenosine transporter. c³Ado did not appear to act through binding to purinergic receptors on EC, since neither ATP, adenosine, nor ADP inhibited thrombin-stimulated PDGF production or monocyte adhesion (Fig. 4).

We have previously demonstrated by Northern blot analysis that thrombin at 12 units/ml and PMA at 10 nM caused maximal increases in the steady-state levels of PDGF-A and...
-B chain mRNA in comparison with the basal level (Fig. 5). c'Ado at 100 μM had a minimal effect on the basal mRNA level of PDGF-B chain; a reduction was, however, observed in the basal expression of PDGF-A chain. The drug, however, significantly reduced the stimulated steady-state levels of both PDGF mRNAs. In addition, c'Ado treatment did not affect the expression of the "housekeeping" gene tubulin. These results indicate that c'Ado does not exert its effect by suppressing transcription nonspecifically. All the agonists tested also strongly induced the steady-state mRNA level of ELAM-1. Unstimulated human aortic EC expressed very low levels of ELAM-1. c'Ado suppressed the stimulation of expression of ELAM-1 mRNA level by thrombin and all other agonists tested without altering tubulin expression (Fig. 6).

Since Northern analysis quantitates the steady-state levels of mRNA, an inhibitory effect of c'Ado could be either due to a decrease in the transcriptional rate or due to destabilization of the induced mRNA. The effect of c'Ado on the transcriptional rate of PDGF and ELAM-1 genes was addressed through nuclear run-on analysis. In the absence of stimulation, very little transcription of either ELAM-1 gene or PDGF-B chain gene was evident (Fig. 7). The basal transcription rate of PDGF-A chain gene in these cells was significantly higher than both PDGF-B chain and ELAM-1 genes. Densitometric analysis correcting for tubulin signal indicated that thrombin caused an increase in the transcriptional rates of the PDGF-A and -B chain genes by 3.5- and 3.8-fold, respectively, as compared with the basal level. The rate of ELAM-1 transcription was increased by approximately 7.5-fold. PMA caused an even greater, 27.1-fold, increase in the transcription of ELAM-1 gene. PMA was also a stronger transcriptional activator of PDGF genes than thrombin. PMA enhanced the transcription of PDGF-A chain gene by 136% and PDGF-B chain gene by 300% above that by thrombin. In the presence of c'Ado alone, the transcription rates of ELAM-1 and PDGF-A and PDGF-B chain genes were similar to the untreated control levels. However, c'Ado proved to be

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**Fig. 3. Transport inhibitor p-nitrobenzylthioinosine reverses the inhibitory effect of c'Ado on PDGF production and monocyte adhesion.** Confluent human aortic EC were preincubated with the indicated concentrations of c'Ado and p-nitrobenzylthioinosine (1 μM) for 30 min at 37 °C in MCDB 107 containing 5% FBS. At the end of the preincubation period, bovine α-thrombin (12 units/ml for PDGF and 30 units/ml for U937 monocytic cell adhesion) was added to wells with p-nitrobenzylthioinosine (●), as well as to wells without p-nitrobenzylthioinosine (■), and the incubation was continued for a further 8 h for PDGF and 6 h for monocytic cell adhesion. c'Ado was present in relevant wells during the entire incubation period. Monolayers were also incubated with c'Ado (100 μM) alone for the entire incubation period (○). PDGF in the conditioned medium (panel A) and U937 monocytic cell adhesion (panel B) were determined as described under "Experimental Procedures." Data represent means ± S.D.; n = 3.

**Fig. 4. Influence of adenosine, ADP, and ATP on basal and thrombin-stimulated PDGF production and monocyte adhesion.** Human aortic EC were incubated with either adenosine (200 μM), ADP (200 μM), or ATP (200 μM) alone or in combination with bovine α-thrombin (Thr) (12 units/ml for PDGF and 30 units/ml for monocyte adhesion) for 24 h for PDGF production and 6 h for U937 monocytic cell adhesion in MCDB 107 with 5% FBS. At the end of the incubation period, PDGF in the conditioned medium and U937 monocytic cell adhesion were measured as described under "Experimental Procedures." Data represent means ± S.D.; n = 3.

**Fig. 5. Effect of c'Ado on thrombin-stimulated PDGF-A and -B chain mRNAs.** Confluent human aortic EC were preincubated with c'Ado (190 μM) for 30 min at 37 °C in MCDB 107 containing 5% FBS. Bovine α-thrombin (12 units/ml) or PMA (10 nm) was added to the appropriate plates, and the incubation was continued for a further 4 h. c'Ado was present in relevant plates during the entire incubation period. Total cellular RNA was isolated, and Northern analysis was performed as described under "Experimental Procedures."
c3Ado was continued for a further 4 h. c3Ado was present in relevant wells during the entire incubation period. Total cellular RNA was isolated from human aortic EC were preincubated with c3Ado (100 μM) for 30 min at 37 °C in MCDB 107 containing 5% FBS. Agonists were added at the indicated concentrations to the appropriate plates, and the incubation was continued for a further 6 h. Antibody binding was quantitated as described under “Experimental Procedures.”

**Fig. 6. Effect of c3Ado on the steady-state level of ELAM-1 mRNA stimulated by different agonists.** Confluent human aortic EC were incubated with c3Ado (100 μM) for 30 min at 37 °C in MCDB 107 containing 5% FBS. Agonists were added at the indicated concentrations to the appropriate plates, and the incubation was continued for a further 6 h. c3Ado was present in relevant wells during the entire incubation period. Total cellular RNA was isolated and Northern analysis was performed as described under “Experimental Procedures.” LPS, lipopolysaccharide.

**FIG. 7. Effect of c3Ado on the transcription rate of PDGF and ELAM-1 genes.** Human aortic EC were incubated in MCDB 107 supplemented with 5% FBS for 3 h at 37 °C under the following conditions. 1, media alone; 2, bovine α-thrombin (12 units/ml for PDGF and 30 units/ml for ELAM-1); 3, PMA (10 nM); 4, c3Ado (100 μM); 5, thrombin and c3Ado; 6, PMA and c3Ado. Nuclei were isolated, and the nuclear run-on assay was performed as described under “Experimental Procedures.”

Our results from monocytic cell adhesion assays and ELAM-1 gene transcription analyses suggested that c3Ado may inhibit agonist-stimulated monocyte adhesion through its ability to prevent the cell surface expression of ELAM-1 on human aortic EC. This possibility was addressed through antibody binding studies. ELAM-1 expression on EC surface was detected by a sandwich technique using the monoclonal antibody 7A9 as the primary anti-ELAM-1 antibody, a biotinylated anti-IgG as the secondary antibody, and [125I]streptavidin as the label binding to the biotin (Fig. 8). In the absence of any agonists, unactivated human aortic EC exhibited very low levels of ELAM-1. Thrombin treatment for 6 h enhanced EC surface expression of ELAM-1 by 3-fold. However, when human aortic EC were treated with thrombin in the presence of c3Ado (200 μM), the expression of ELAM-1 was reduced to levels very similar to that seen on unactivated human aortic EC (media control). c3Ado treatment by itself did not affect ELAM-1 expression. The specificity of the primary antibody was assessed by the inclusion of a type (IgG1)-matched nonspecific myeloma protein (MOPC21). Experiments with MOPC21 confirmed the ability of the monoclonal antibody 7A9 to specifically bind to ELAM-1. These results further demonstrate that thrombin stimulates U937 cell adhesion through the expression of ELAM-1 adhesion molecules, and c3Ado inhibits thrombin-stimulated U937 cell adhesion by virtue of its ability to block the EC surface expression of ELAM-1.

**DISCUSSION**

Activation of the endothelium by humoral agonists may play an important role in the physiology and pathology of the vessel wall. Such an activation process may lead to thrombus formation on the arterial surface. Within the microenvironment of the mural thrombi, the protease thrombin may be found in sufficient concentrations to act as a potent EC agonist. Monocyte adhesion and PDGF production are two events that may be stimulated by thrombin under these conditions. Our studies demonstrate that the drug c3Ado is effective in preventing both thrombin-stimulated PDGF production and monocyte adhesion.

The inhibitory influence of c3Ado on EC production of PDGF appears to be specific for agonist-induced expression. This drug does not alter constitutive PDGF production by human aortic EC. Our data indicate that c3Ado inhibits the thrombin-stimulated transcriptional rate of both the PDGF-A and-B chain genes. Furthermore, the action of c3Ado is not due to nonspecific cellular toxicity since overall protein synthesis of the cells and the rate of transcription of a housekeeping gene was unaltered during the incubation period.

We have previously established that the monocytic cell line U937 serves as a model for blood-borne monocytes in studies of adhesion to EC (16, 23). Thrombin and other agonists, such as IL-1β and PMA, are effective stimulators of monocyte adhesion to human aortic EC. This in vitro adhesion process appears to be mediated in part through the expression of the EC surface receptor, ELAM-1 (27). c3Ado was effective in
c'Ado Inhibits Thrombin-stimulated ELAM-1 and PDGF Expression

References

36. Yagawa, K., Nakamishi, M., Hayashi, S., Kaku, M., Ichinose, Y.,
cAdo Inhibits Thrombin-stimulated ELAM-1 and PDGF Expression