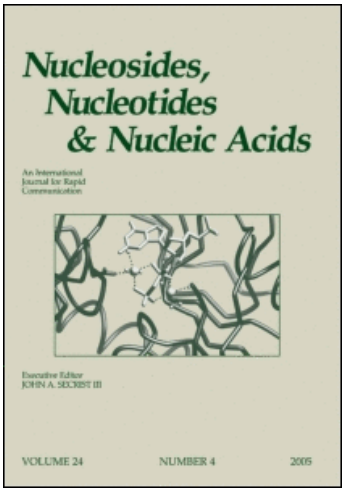


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# Anti-Inflammatory Activity of Purine Nucleoside Analogs

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# ANTI-INFLAMMATORY ACTIVITY OF PURINE NUCLEOSIDE ANALOGS

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## ABSTRACT

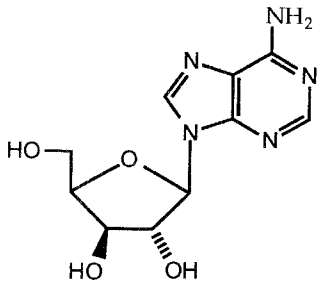
It is well known that adenosine plays an important role in inflammatory processes. A collection of adenosine analogs modified in the base and/or sugar functional moiety have been synthesized and submitted for biological testing. Each purine nucleoside analog was tested for inhibition of endothelial cell activation by various inflammatory stimuli. A number of analogs exhibited potent anti-inflammatory activity. Animal studies have been carried out on AMG002370 which was found to potently inhibit adjuvant induced arthritis in the Lewis rat.

## INTRODUCTION

A collection of adenosine analogs have been examined for their ability to inhibit cytokine (IL-8) production from human umbilical vein endothelial cells (HUVEC) induced by various inflammatory stimuli. AMG002370 (Figure I) significantly inhibited ($IC_{50}$ = 0.5μM) both TNFα and LPS stimulated IL-8 production while having no effect on cell viability at concentrations up to 100μM (Figure II). In addition, AMG002370 was found to inhibit the TNFα-induced expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) in a dose dependent fashion. Interestingly, AMG002370 does not effect the TNFα-induced activation of the transcription factor NF-κB, an important regulator of adhesion molecule gene expression. In an effort to unveil important structure activity relationships and help determine the biochemical mechanism of action, over thirty-five analogs modified in the base and/or sugar moiety were synthesized and tested.

Studies have shown that the activity of AMG002370 is dependent on nucleoside transport. S-(p-nitrobenzyl)-6-thioinosine is a potent inhibitor of nucleoside transport, but has little effect on membrane signalling events. AMG002370 is inactive when S-(p-nitrobenzyl)-6-thioinosine is present (data not shown). The blockade of AMG002370 by this nucleoside transport inhibitor indicates that cellular uptake is crucial for inhibitory effects.

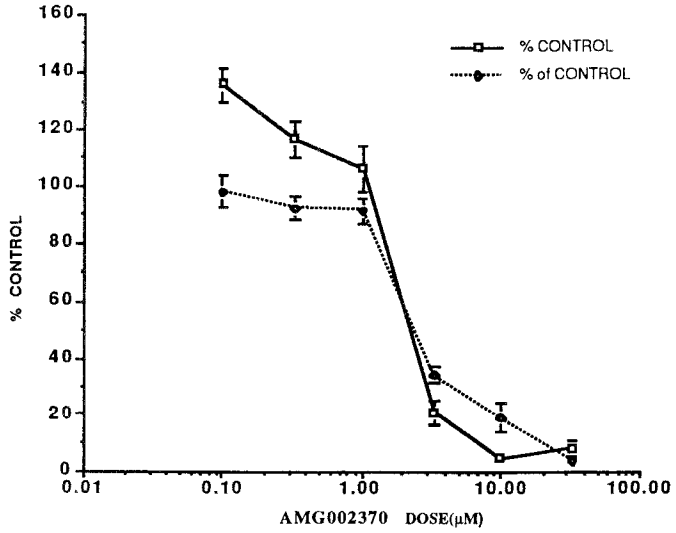
AMG002370 had no effect on NF-κB activation and IκB-α degradation in TNFα - stimulated cells (data not shown). Numerous studies have demonstrated that NF-kB is required for cytokine- and endotoxin-induced expression of CAM genes (E-selectin, VCAM-1 and ICAM-1)[1]. Treatment of HUVEC with TNFα resulted in rapid appearance of NF-κB/DNA-binding activity. Pretreatment of HUVEC with adenosine (10μM) prior to TNFα stimulation had no effect on this activity. Interestingly, AMG002370 (10μM) also had no effect on NF-κB activation in TNFα-stimulated HUVEC. In HUVEC, the TNFα-induced appearance of nuclear NF-κB/DNA-binding coincides with the disappearance of IκB-α. Activation of HUVEC with TNFα results in the complete loss of IκB-α from cytoplasm. Pretreatment of HUVEC with either adenosine (10μM) or AMG002370 (10μM) did not prevent the TNFα-induced degradation of IκB-α. (data not shown).




AMG002370

**Figure I**

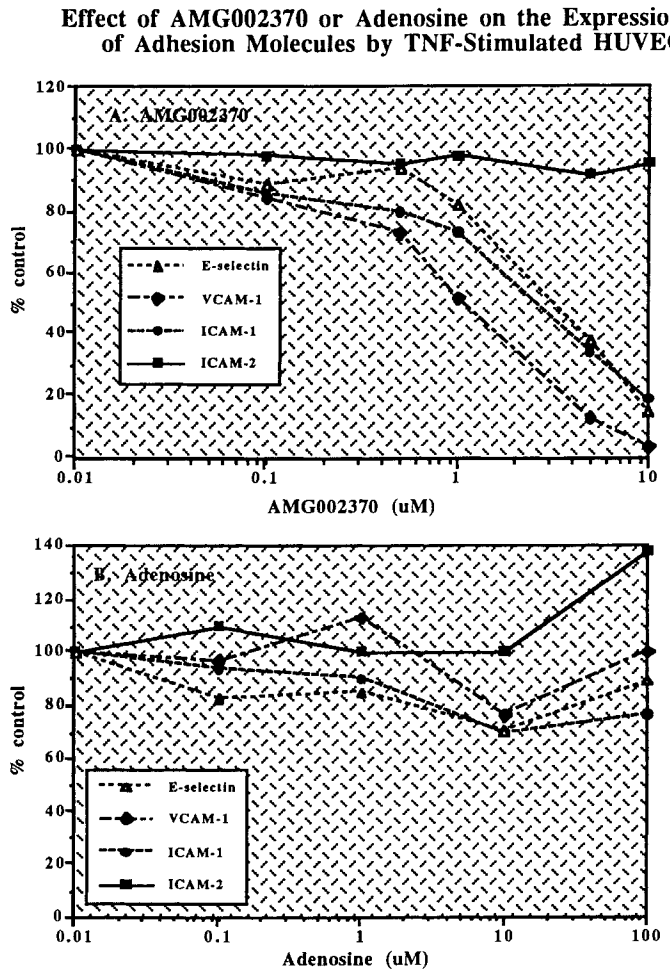





AMG002370 INHIBITION OF IL-8 PRODUCTION FROM HUVECS IN RESPONSE TO TNFα (0.01ug/ml) AND LPS (0.1ug/ml) STIMULI

**Figure II**

The data shown in Figure II was obtained as follows. AMG002370 and adenosine were added to HUVECs. Endothelial basal media (EMB) was added to unstimulated and stimulated controls. The stimuli is added ( TNF-α or LPS ) at 10X concentration to each sample excluding the unstimulated control. After incubation the samples were centrifuged and the supernatant was isolated for IL-8 determination.




## Figure III

**Inhibition of TNFa-induced cell surface expression of E-selectin, VCAM-1, ICAM-1 and ICAM-2 by AMG002370 in endothelial cells.**

HUVEC monolayers in 96-well plates were either left untreated or pretreated with various concentration of AMG002370 (A) or adenosine (B) for 30-60 minutes before stimulation with TNFα (10 ng/ml). Test and control samples were performed in triplicate in each experiment. The degree of specific antibody binding was calculated by subtracting the mean negative control value from each test value, and results were expressed as a percentage of the TNFα-treated control values. In all cases, observed SD were less than 5% of mean values. Data from a single experiment are shown, which are representative of four similar experiments.

## CONCLUSION

By randomly screening several existing intermediates used in nucleic acid analog synthesis, a potent inhibitor of TNF signaling in endothelial cells was identified. The high nanomolar activity of this compound in a cell culture assay led to a preliminary animal model evaluation. Although animal studies demonstrated efficacy, weight loss was observed as well as other potential toxicity. In addition, the potential interference with several important cellular processes as well as the metabolic instability of this class of compounds limits their therapeutic utility. Perhaps the most interesting result of this study is the identification of a novel mechanism of inhibition of TNF and LPS signaling in endothelial cells that appears to be independent of NF-*k*B activation.

## REFERENCE

[1] Collins, T., Palmer, H.J., Whitley, M.Z., Neish, A.S., and Williams, A.J., *Trends Card. Med* 1993 3 (92)