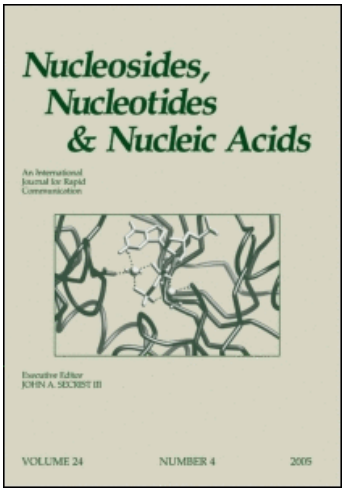

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PURINE METABOLISM AND CLINICAL STATUS OF PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH DIPYRIDAMOLE

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□ *The anti-inflammatory activities of methotrexate and sulphasalazine may be mediated by increases in endogenous adenosine levels. Since the vascular protective drug dipyridamole inhibits the uptake and metabolism of adenosine we have now tested this compound in patients with rheumatoid arthritis to assess its effects on their symptoms. Forty patients (aged 18–75 years) received dipyridamole 400 mg/day or placebo. The levels of adenosine and its major metabolites were determined by high performance liquid chromatography (HPLC) in blood samples taken at baseline and at monthly intervals during treatment for 6 months. After three months of treatment there was a significant reduction in the modified Health Assessment Questionnaire (mHAQ) score, but these effects were not maintained, and dipyridamole did not modify disease severity scores or the levels of adenosine and its metabolites. We conclude that the symptoms of rheumatoid arthritis were not modified by treatment with dipyridamole.*

Keywords Arthritis; Dipyridamole; Adenosine; Cytokines

INTRODUCTION

It has been suggested[1] that part of the anti-inflammatory effects of methotrexate and sulphasalazine—disease-modifying anti-rheumatoid drugs—may be mediated by the release of endogenous adenosine since this nucleoside can modify the release of inflammatory mediators or reactive oxygen species from immune-competent cells.[2,3] The concept of a key role for adenosine receptors in inflammatory processes is supported by the upregulation, especially of A2A and A2B receptors, that occurs in activated monocytes and macrophages.

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Dipyridamole is well established in the treatment of angina pectoris and cerebrovascular disease, acting primarily by inhibiting cellular uptake of adenosine and thereby producing vasodilatory and anti-aggregatory effects. We have now investigated whether dipyridamole can increase adenosine levels in rheumatoid patients, to an extent that could ameliorate the symptoms of rheumatoid arthritis.

MATERIALS AND METHODS

Patients and Recruitment. Forty patients (aged 18–75 years), were recruited from routine rheumatology clinics (LGD), and rheumatoid arthritis was diagnosed using the American Rheumatism Association revised criteria.[4] Patients were randomized to receive dipyridamole (200 mg twice daily; n = 21) or placebo (n = 19) in addition to their usual antirheumatic therapy if this comprised nonsteroidal anti-inflammatory drugs, prednisolone (up to 7.5 mg/day) or slowly-acting antirheumatic drugs taken at a constant dosage for at least 6 months. All patients gave written, informed consent to participation. A clinical assessment was undertaken at monthly intervals and included tender joint count, swollen joint count, assessment of joint movement, patient's assessment of pain, patient's and physician's global assessment of disease activity, and a modified Health Assessment Questionnaire (MHAQ). Blood samples were taken at baseline before treatment, and at monthly intervals during treatment for 6 months. Blood was always collected in the morning.

Analysis of Purines by HPLC. Serum samples were acidified with 4 M perchloric acid, vortexed and centrifuged at 5,000 g for 10 minutes, this process being repeated 3 times and the supernatants combined, filtered, and centrifuged using Whatman Vectaspin Micro Anopore tubes. A Waters HPLC system was used with a Phenomenex Synergi-Fusion-RP column at 25°C, a flow rate of 0.8 ml/min and a mobile phase of 2.5 mM ammonium dihydrogen orthophosphate buffered to pH3.5 using 5% phosphoric acid. A gradient increased acetonitrile from zero to 6% between 20 and 30 minutes, with the 6% acetonitrile mobile phase being maintained for a further 10 minutes. Samples were detected by a UV detector at 254 nm.

Statistics. Data are expressed as mean ± 1 SEM. Analysis of variance (ANOVA) followed by Dunnett's posttest was used for multiple comparisons of monthly time points with baseline. Where a non-parametric analysis was required, ANOVA was followed by Dunn's multiple comparisons test. Comparisons between placebo and treatment values at individual time points were made using an unpaired t test or, where a nonparametric analysis was required, a Mann-Whitney test.

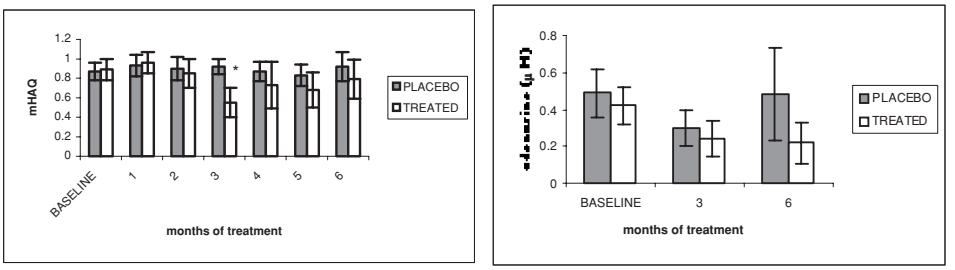

FIGURE 1 Histograms of the Health Assessment Questionnaire scores and concentrations of adenosine in the plasma of patients with rheumatoid arthritis, before and during treatment with dipyridamole compared with patients receiving a placebo. There were significant changes ($p < 0.05$) only at the 3-month time point of the HAQ score, but not for adenosine levels or any of its metabolites.

RESULTS

Dipyridamole did not change patients' symptoms as indicated by the clinician's rheumatoid arthritis severity score. A significant reduction in the modified Health Assessment Questionnaire (mHAQ) score was observed, compared with placebo, after 3 months of treatment with dipyridamole but this difference was not maintained subsequently (Figure 1). This may have been due to the withdrawal of several patients because of side effects such as hypotension and headaches. This resulted in greater standard errors which may have biased the data.

Adenosine and Purines. The levels of adenosine (Figure 1) and its major metabolites inosine, xanthine, hypoxanthine, and uric acid (not illustrated) were unchanged by treatment with dipyridamole throughout the period of this study.

DISCUSSION

Adenosine can modify the production of pro-inflammatory cytokines from activated immune-competent cells[5–8] and inhibits the oxidative respiratory burst which generates free radicals that can contribute to joint tissue damage in arthritis.[9,10] Some disease-modifying drugs, such as methotrexate and sulphasalazine may work partly by the release of adenosine, although this concept has not been well investigated in patients.

Several factors may account for the absence of clear changes of symptom severity or purine levels. The dose of dipyridamole may have been too high for some patients to tolerate, since several patients withdrew because of unacceptable side effects. Pharmacokinetic parameters may also be relevant. Dipyridamole has an α-t $^1/_2$ (distribution half-life) of around 40 minutes in humans, and plasma levels may not have been maintained high enough to inhibit adenosine uptake sufficiently. Adenosine is taken up rapidly by tissues and a high, constant level of dipyridamole may be

necessary to produce an adequate suppression of this removal. It could be argued that the efficacy of dipyridamole in cerebrovascular disease indicates that blood levels of the drug are adequate to raise adenosine levels and that pharmacokinetic factors are not relevant. However, dipyridamole also inhibits cyclic nucleotide phosphodiesterase and suppresses the formation of thromboxane A2. Since both the A1 and A2A adenosine receptors act partly via adenylate cyclase, the inhibition of phosphodiesterase almost certainly will modify these actions and, as a result, vascular patency and platelet aggregation.

Overall the results indicate that, although there is clear evidence that adenosine can modulate the production and release of pro-inflammatory mediators and free radicals, the use of dipyridamole to raise blood purine levels is not adequate to elevate them sufficiently, or consistently enough, to produce any significant change of symptoms in patients with rheumatoid arthritis.

REFERENCES

1. Cronstein, B.N.; Bouma, M.G.; Becker, B.F. Purinergic mechanisms in inflammation. *Drug Dev. Res.* **1996**, 39, 426–435.
2. Cronstein, B.N.; Rosenstein, E.D.; Kramer, S.B.; Weissmann, G.; Hirschhorn, R. Adenosine: a physiological modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. *J. Immunol.* **1985**, 135, 1366–1371.
3. Gessi, S.; Varani, K.; Merighi, S.; Ongini, E.; Borea, P.A. A(2A) adenosine receptors in human peripheral blood cells. *Brit. J. Pharmacol.* **2000**, 129, 2–11.
4. Arnett, F.C.; Edworthy, S.M.; Bloch, D.A.; Mcshane, D.J.; Fries, J.F.; Cooper, N.S.; Healey, L.A.; Kaplan, S.R.; Liang, M.H.; Luthra, H.S.; Medsger, T.A.; Mitchell, D.M.; Neustadt, D.H.; Pinals, R.S.; Schaller, J.G.; Sharp, J.T.; Wilder, R.L.; Hunder, G.G. The American-rheumatism-association 1987 revised criteria for the classification of rheumatoid-arthritis. *Arthritis & Rheumatism* **1988**, 31, 315–324.
5. Cronstein, B.N. Adenosine, an endogenous antiinflammatory agent. *J. Appl. Physiol.* **1994**, 76, 5–13.
6. Bshesh, K.; Zhao, B.; Spight, D.; Biaggioni, I.; Feokistov, I.; Denenberg, A.; Wong, H.R.; Shanley, T.P. The A2A receptor mediates an endogenous regulatory pathway of cytokine expression in THP-1 cells. *J. Leuk. Biol.* **2002**, 72, 1027–1036.
7. Hasko, G.; Kuhel, D.G.; Chen, J.F.; Schwarzschild, M.A.; Deitch, E.A.; Mabley, J.G.; Marton, A.; Szabo, C. Adenosine inhibits IL-12 and TNF-alpha production via adenosine A(2a) receptor-dependent and independent mechanisms. *FASEB J.* **2000**, 14, 2065–2074.
8. Salmon, H.E.; Cronstein, B.N. Fcγ receptor-mediated functions in neutrophils are modulated by adenosine receptor occupancy: A1 receptors are stimulatory and A2 receptors are inhibitory. *J. Immunol.* **1990**, 145, 2235–2240.
9. Hannon, J.P.; Bray-French, K.M.; Phillips, R.M.; Fozard, J.R. Further Pharmacological characterization of the adenosine receptor subtype mediating inhibition of oxidative burst in human isolated neutrophils. *Drug Develop. Res.* **1998**, 43, 214–224.
10. Revan, S.; Montesinos, M.C.; Naime, D.; Landau, S.; Cronstein, B.N. Adenosine A2 receptor occupancy regulates stimulated neutrophil function via activation of a serine/threonine protein phosphatase. *J. Biol. Chem.* **1996**, 271, 17114–17118.