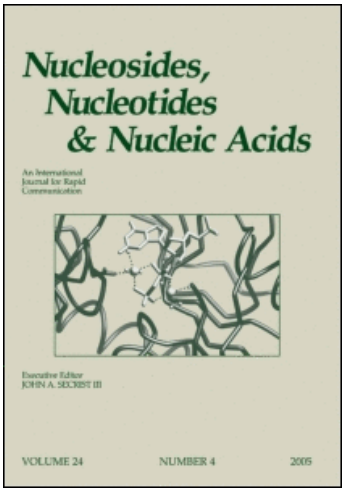

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Purine Modulation of Cytokine Release During Diuretic Therapy of Rheumatoid Arthritis

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ABSTRACT

Since free radicals are implicated in rheumatoid arthritis (RA) and since uric acid is a free radical scavenger, we examined the effects of treating RA patients with with the diuretic bumetanide to try to improve their arthritic control. Seventy patients, aged 18–75 years, were randomised to receive bumetanide 4 mg/day or placebo. Uric acid levels increased, but not that of other purines, in the blood of drug-treated patients compared with placebo-treated controls. There were no significant changes in clinical measurements of disease activity or in ESR or CRP levels. There were no over all differences in the blood levels of the cytokines, nor in the basal or stimulated production of cytokines from the blood cultures. The adenosine receptor agonist 5′Nethylcarboxamido- adenosine (NECA) used to modify cytokine release in cultures of whole blood taken from the patients, depressed the release of tumour necrosis factor-a (TNFa), but failed to depress the release of interleukin-1b (IL-1b) or interleukin-6 (IL-6), a difference from earlier studies of healthy control subjects and, thus, a difference which may contribute to the disease activity.

Key Words: Rheumatoid arthritis; Cytokines; Uric acid; Free radicals; Adenosine receptors; Bumetanide.

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INTRODUCTION

Free radicals (FR) may be involved in the destruction of joint tissues in rheumatoid arthritis (RA). Cytokines such as tumour necrosis factor-α (TNF-α) increase FR formation in white cells and synovial tissue, whereas uric acid is a powerful FR scavenger.[1] Since several diuretics raise blood levels of uric acid, the present study was initiated to determine whether treatment with the oral diuretic bumetanide could raise serum uric acid levels in patients with RA to an extent which would ameliorate inflammation by cytokine modulating and anti-oxidant effects. We have also measured the levels of purine precursors of urate, and the ability of adenosine receptors to modulate stimulated cytokine release in whole blood cultures.

METHODS

Seventy patients (aged 18–75 years) were recruited and RA was diagnosed using the American Rheumatism Association revised criteria.[2] Patients were excluded if taking any drug which affects blood uric acid levels. Patients gave written, informed consent and were randomised to receive bumetanide 4 mg/day or identical placebo pills. Full clinical assessment was undertaken using the measures of disease activity from the American College of Rheumatology. Patients were assessed and blood taken at monthly intervals for 6 months. The study was conducted with the full approval of the District Medical Ethics Committee.

Serum was stored at −70°C until required for assay or diluted with medium for cell culture experiments. To stimulate cytokine release, lipopolysaccharide (LPS, from *Salmonella typhimurium*) was added (100 ng/ml). 5′N-ethylcarboxamido-adenosine (NECA, 2 μM) was used as a non-selective adenosine receptor agonist. Cultures were incubated for 40 h at 37°C in 5% CO_2, and were then centrifuged and stored at −70°C. Purine levels (adenosine, inosine, xanthine, urate) were measured by HPLC using UV detection at 254 nm. Cytokines and neopterin were measured using commercial ELISA

Table 1. Levels of uric acid and purine metabolites in the blood of placebo and bumetanide-treated patients with RA.

Month of treatment	Placebo control patients	Drug-treated patients
Baseline	254.6 ± 21.5	265.3 ± 19.1
1	247.8 ± 15.8	312.4 ± 223.3*
2	260.4 ± 19.8	372.9 ± 22***
3	232.7 ± 23.4	322.8 ± 22.1**
4	226.5 ± 25.7	314.7 ± 21.8*
5	225.4 ± 22.8	328.6 ± 32.4*
6	300.4 ± 58.4	312.2 ± 49.6

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$ compared with the placebo controls.

Table 2. Effects of NECA on the basal and LPS-stimulated release of TNF-α from whole blood cultures.

Month of treatment	Basal release	NECA 2 μM	LPS	LPS + NECA
Placebo controls				
Baseline	515.2 ± 88.6	90.5 ± 25.5***	612.3 ± 94.4	131.76 ± 22.4***
1	488.6 ± 69.3	114.5 ± 24.3***	622.6 ± 78.5	144.0 ± 26.8***
6	404.5 ± 60.7	87.5 ± 39.6***	611.3 ± 94.5	102.4 ± 33.8***
Drug-treated				
Baseline	532.6 ± 64.5	110.3 ± 22.1***	665.7 ± 72.6	210.2 ± 24.5***
1	415.4 ± 144.6	122.4 ± 37***	616.6 ± 175.4	142.8 ± 32.6***
6	457.9 ± 72.3	55.4 ± 29***	795.0 ± 198.5	162.4 ± 43.8***

***$p < 0.001$ compared with the placebo controls.

kits, and lipid peroxidation products were assayed using the Bioxytech LPO-586 colorimetric assay (R&D systems).

RESULTS

Uric acid levels were significantly higher in drug-treated patients compared with placebo controls at each monthly sampling point (Table 1), but no consistent change was seen in the levels of inosine, adenosine, hypoxanthine or xanthine. None of the clinical parameters or biochemical correlates such as ESR and neopterin levels showed a significant change over the time course of the study.

Similarly serum levels of TNF-α, IL-1β and IL-6 remained unchanged during the study, and showed no differences between bumetanide and placebo-treated patients. The release of TNF-α by LPS was reduced in the presence of the adenosine receptor agonist NECA (2 μM) in cultures from patients or controls, with no significant differences between patient and control blood samples (Table 2). Although LPS increased the release of IL-1β and IL-6, NECA did not suppress their production.

DISCUSSION

Agudelo et al.[3] proposed that persistent hyperuricaemia might protect against rheumatoid inflammation and it has been suggested that uric acid is the most important antioxidant in patients with RA,[4] although we been unable to confirm this.[5] We have confirmed that bumetanide treatment raised blood levels of urate[6] but, since the levels of other purines were unchanged, this effect may result from changes of renal transport processes,[7] rather than of purine metabolism.

The release of pro-inflammatory cytokines from activated macrophages and neutrophils can be modulated by purine receptors,[8] and we reasoned that a diuretic-induced increase in plasma adenosine might suppress cytokine release and improve rheumatoid symptomatology. The absence of any difference between the effects of

NECA on TNF-α release supports the view that bumetanide does not alter purine metabolism or the sensitivity of purine receptors.

Although we were able to confirm that NECA could suppress the basal or LPS-stimulated production of TNF-α in whole blood cultures, NECA failed to modify the release of IL-1β or IL-6 in any of the RA patient populations. Adenosine receptors can inhibit LPS-stimulated IL-6 release in blood cultured from healthy volunteers.[9] We conclude that adenosine receptors are less able to suppress interleukin release in RA patients than in healthy subjects. This loss of tissue protection by adenosine may contribute to disease activity.

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