

Allopurinol Use Yields Potentially Beneficial Effects on Inflammatory Indices in Those With Recent Ischemic Stroke

A Randomized, Double-Blind, Placebo-Controlled Trial

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Background and Purpose—Elevated serum uric acid level is associated with poor outcome and increased risk of recurrent events after stroke. The xanthine oxidase inhibitor allopurinol lowers uric acid but also attenuates expression of inflammatory adhesion molecules in murine models, reduces oxidative stress in the vasculature, and improves endothelial function. We sought to investigate whether allopurinol alters expression of inflammatory markers after acute ischemic stroke.

Methods—We performed a randomized, double-blind, placebo-controlled trial to investigate the safety, tolerability, and effect of 6 weeks' treatment with high- (300 mg once a day) or low- (100 mg once a day) dose allopurinol on levels of uric acid and circulating inflammatory markers after ischemic stroke.

Results—We enrolled 50 patients with acute ischemic stroke (17, 17, and 16 in the high, low, and placebo groups, respectively). Mean (\pm SD) age was 70 (\pm 13) years. Groups had similar characteristics at baseline. There were no serious adverse events. Uric acid levels were significantly reduced at both 7 days and 6 weeks in the high-dose group (by 0.14 mmol/L at 6 weeks, $P=0.002$). Intercellular adhesion molecule-1 concentration (ng/mL) rose by 51.2 in the placebo group, rose slightly (by 10.6) in the low-dose allopurinol group, but fell in the high-dose group (by 2.6; difference between groups $P=0.012$, Kruskal-Wallis test).

Conclusion—Allopurinol treatment is well tolerated and attenuates the rise in intercellular adhesion molecule-1 levels seen after stroke. Uric acid levels were lowered with high doses. These findings support further evaluation of allopurinol as a preventive measure after stroke. (*Stroke*. 2008;39:3303-3307.)

Key Words: stroke management ■ uric acid ■ xanthine oxidase

A wealth of epidemiological data shows that elevated serum uric acid (UA) level is associated with an increased risk of vascular events,¹ including in the poststroke period.² An association between elevated UA and worse outcome after stroke has been observed,^{1,3} although discordant reports exist.⁴ The uricosuric effects of the angiotensin receptor blocker losartan may contribute to its greater benefit compared with atenolol with regard to stroke reduction.⁵ Furthermore, use of xanthine oxidase inhibition may yield additional benefits in addition to potent UA reduction. Allopurinol, the most commonly used xanthine oxidase inhibitor, reduces oxidative stress in the vasculature,⁶ improves endothelial function in a variety of cardiovascular disease states,⁷ and reduces expression of proinflammatory molecules such as soluble intercellular adhesion molecule-1 (ICAM-1) in vitro.⁸ Allopurinol has not yet been studied in the poststroke setting.

We investigated the effect of allopurinol on levels of UA and circulating inflammatory markers in a group of patients with recent ischemic stroke.

Methods

We performed a prospective randomized, double-blind, placebo-controlled study. Patients were recruited within 72 hours of radiologically confirmed ischemic stroke. Exclusion criteria were a contraindication to allopurinol treatment, serum creatinine concentration >200 μ mol/L, concurrent warfarin treatment, and comorbidity likely to cause death within 3 months. Patients were randomized to receive either high-dose (300 mg) or low-dose (100 mg) allopurinol or matching placebo on a 1:1:1 basis for 6 weeks. The randomization code was kept in the pharmacy department of the Western Infirmary Hospital and was not broken until all follow-up was complete. Those with clinically apparent infection, subsequently corroborated with elevated C-reactive protein, were excluded from the analyses of inflammatory parameters (this was decided before analysis and unblinding of the study).

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Table 1. Demographic and Baseline Variables

	Placebo	Low Dose	High Dose
Age, years, mean (SD)	69 (14)	70 (14)	71 (11)
Male sex, no. (%)	6 (46)	6 (43%)	6 (38%)
Stroke subtype, no. (%)			
PACS	7 (44%)	11 (65%)	7 (41%)
LACS	8 (50%)	6 (35%)	9 (53%)
POCS	1 (6%)	0	1 (6%)
Thiazide use	3 (23%)	4 (29%)	2 (12%)
Diabetes	3 (23%)	3 (21%)	0
Baseline UA, mmol/L	0.34	0.34	0.35
Baseline ICAM-1, ng/mL	206.7 (167.4–255.2)	217 (193.9–297.6)	250.2 (159.9–322.1)
Baseline CRP, mg/L	3.59 (2.37–6.25)	1.61 (1.26–18.11)	3.75 (1–11.45)
Baseline IL-6, pg/mL	4.23 (1.87–9.09)	4.09 (2.2–11.61)	4.02 (3.34–5.9)

Values shown are median (interquartile range) unless otherwise stated. The baseline levels of inflammatory markers shown exclude those with elevated baseline CRP and clinical evidence of infection.

PACS indicates partial anterior circulation syndrome; LACS, lacunar syndrome; POCS, posterior circulation syndrome.

The study was approved by the local research ethics committee. All participants gave written informed consent. All study visits took place in the Acute Stroke Unit at the Western Infirmary Hospital Glasgow.

Demographic details and assessment of stroke severity were obtained at baseline and blood was drawn to measure UA, ICAM, C-reactive protein (CRP) and interleukin-6 (IL-6) levels. This was repeated at 1 and 6 weeks from randomization.

ICAM-1 was measured in citrated plasma stored at -80°C using high-sensitivity commercial enzyme-linked immunosorbent assay kits (R&D Systems). CRP was measured using a high-sensitivity, 2-site enzyme-linked immunoassay. IL-6 was measured by a high-sensitivity commercial enzyme-linked immunosorbent assay (R&D Systems).

Statistical Analysis

Our primary end point was change in UA levels at 6 weeks. Secondary end points were change in ICAM-1, CRP, and IL-6 levels at 1 and 6 weeks. We calculated that 13 subjects per group would be required to detect a 0.18-mmol/L difference in UA concentration between groups with 90% power. We assumed a SD of 0.13 mmol/L in UA levels. We anticipated a 10% to 15% dropout rate and further losses due to mortality so we aimed to recruit 50 patients in total. Analysis of variance, 2-sample *t* tests, or their nonparametric equivalents were used for all statistical analyses (Minitab v15; Minitab Inc). A general linear model was applied to adjust for differences in baseline levels of key variables.

Results

In total, 50 patients were recruited (17 each in the high- and low-dose groups and 16 in the placebo group). Half were male, mean (\pm SD) age was 70 (\pm 13) years, and median baseline National Institutes of Health Stroke Scale score was 3 (interquartile range, 2 to 4). Groups were well matched. Demographic details, including baseline UA, ICAM-1, CRP, and IL-6, are displayed in Table 1.

No serious adverse events occurred. Two patients stopped treatment in each of the low-dose and placebo groups. In each group, one stopped treatment because of gastrointestinal upset and one due to the required introduction of warfarin therapy. One patient stopped treatment in the high-dose

allopurinol group due to requirement for azathioprine therapy (deemed a contraindication to allopurinol use). All those in the high-dose group completed follow-up, although one patient did not have a 6-week UA level measured. One patient in the low-dose group and 3 patients in the placebo group (including the 2 withdrawals from treatment) did not complete follow-up (Figure).

Of those who completed follow-up, 3 patients from the placebo group, 2 from the low-dose group, and one from the high-dose group were excluded from analyses of change in inflammatory markers (clinically apparent infection and baseline CRP of >20 mg/L for all).

Uric Acid Levels

UA levels at baseline and 6 weeks are shown for all groups in Table 2. Average UA levels changed little in the placebo and low-dose groups but fell significantly in the high-dose group by 0.14 mmol/L at 6 weeks ($P=0.002$ for difference between groups on Kruskal-Wallis test; 95% CI for the difference between the high-dose and placebo groups -0.19 mmol/L to -0.06 mmol/L on Mann-Whitney test).

Intercellular Adhesion Molecule-1 Levels

Mean ICAM-1 concentration rose by 51.2 ng/mL in the placebo group, rose slightly (by 10.6 ng/mL) in the low-dose allopurinol group, but fell in the high-dose group (by -2.6 ng/mL; difference between groups $P=0.012$, Kruskal-Wallis test; Table 2). A general linear model was applied to adjust for differences in baseline ICAM-1 concentration and showed that the differences between groups persisted and that baseline ICAM-1 levels were not associated with change in ICAM-1 level ($P=0.028$ for differences between groups and $P=0.24$ for differences in baseline ICAM-1 level). There were no statistically significant differences between groups at 1 week (data not shown). When all patients, including those with clinical infection and elevated baseline CRP were included in a post hoc sensitivity analysis, the median

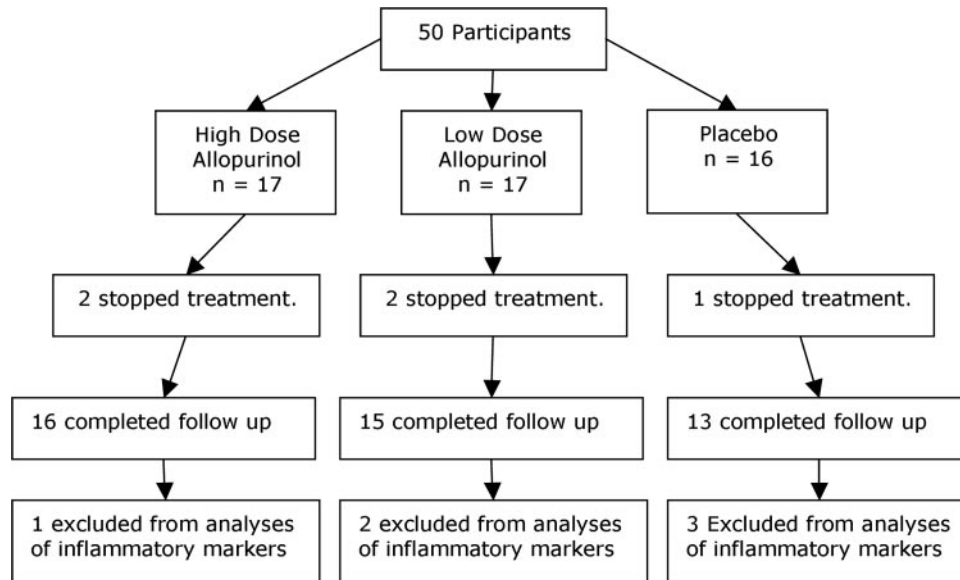


Figure. Randomization and patient flow chart.

changes seen in ICAM-1 levels were similar (41.79, 2.54, and -2.66 ng/mL in the placebo, low- and high-dose groups, respectively), but the difference did not reach statistical significance ($P=0.213$ on Kruskal-Wallis test). Three patients in the placebo group had markedly elevated baseline CRP levels (165.8, 3099, and 37.1 ng/mL, respectively) and had among the highest levels of soluble ICAM-1 and thus the largest falls.

C-Reactive Protein and Interleukin-6 Levels

CRP and IL-6 levels did not change in any group and there were no significant differences between groups (Table 2).

Stroke Severity

Median change in National Institutes of Health Stroke Scale score at 6 weeks was -1 in all groups ($P=0.3$ on Kruskal-Wallis test).

Discussion

Our results suggest that allopurinol attenuates the rise in the proinflammatory marker soluble ICAM-1 seen after acute ischemic stroke. This is the first evidence of a beneficial effect of allopurinol on surrogate markers of vascular and metabolic health after stroke. Our results also show that to achieve sustained reduction in serum UA in the poststroke period, a 300-mg dose of allopurinol is required. We saw a 0.14-mmol/L reduction in serum UA, which is well within a potentially beneficial range.^{1,2}

A number of large, well-conducted epidemiological studies support the hypothesis that elevated serum UA is a powerful predictor of increased risk of cardiovascular event rate and mortality.^{1,7} Small increments in UA are associated with reduced likelihood of favorable 90-day outcome and increased risk of recurrent vascular events,^{2,3} although others hypothesize that the antioxidant effects of increasing UA are associated with improved outcome in the peri-infarct period.⁴ Furthermore, a small study of 24 patients has recently shown that administration of UA within hours of ictus in those treated with thrombolytic therapy for ischemic stroke yields lower lipid peroxidation.⁹ This potential conflict with our data is interesting and warrants further evaluation because at present, no firm conclusions can be drawn. It is entirely plausible that chronic elevations in serum UA could convey harm but that the potential antioxidant effects of UA itself can be harnessed in acute ischemia and oxidative stress. However, as much, if not more, data exist to support pursuit of UA-lowering strategies after stroke. Data exist to support harmful effects of UA on platelet, smooth muscle, and endothelial function.⁷ Equally, recent post hoc analyses of the Losartan Intervention For End point reduction (LIFE) study suggest UA reduction impacts favorably on cardiovascular risk. Perhaps more convincingly, interventional studies of xanthine oxidase inhibition, which will reduce both UA and oxidative stress, consistently show benefits on measures of endothelial function in patients with or at high risk of vascular disease.⁷ This is of interest because several drugs are routinely used to lower UA in other conditions.

However, the previously witnessed effect of allopurinol may relate more to xanthine oxidase inhibition per se and a reduction in oxidative stress than to UA reduction.¹⁰ It is of course possible that elevated UA may signal harm as an indirect marker of greater pro-oxidative stress rather than being a direct toxic agent. Clearly, more studies are needed to disentangle the causal mechanisms, but our data support this; we saw little impact on UA levels with low-dose allopurinol

Table 2. Change in Blood Markers at 6 Weeks

	Placebo	Low Dose	High Dose	P Value
Change in UA	0.02	-0.02	-0.14	0.002
Change in ICAM-1	51.2	10.6	-2.6	0.012
Change in CRP	-2.33	-0.48	-0.28	0.9
Change in IL-6	-1.52	-0.80	-0.36	0.5

Kruskal-Wallis test was used to evaluate median change in parameters.

but saw a similar, albeit smaller, effect on inflammatory markers to that of high dose. Importantly, however, it remains possible that reducing UA will yield benefits through, for example, changes in smooth muscle function in the longer term. Either way, our data show allopurinol may be of use and can be used safely in the early postinfarct period.

We did not see any differences in neurological recovery, as suggested by some animal work.¹¹ However, we would not expect to detect neurological benefit in a small pilot study in which patients were randomized up to 3 days after ictus and were so mildly affected pretreatment.

Allopurinol has recently been shown to inhibit expression of ICAM-1 *in vitro*.⁸ Our study is the first to evaluate this in patients with acute stroke. Soluble ICAM-1 expression is increased when endothelium is activated or damaged and levels are known to rise after acute stroke,^{12,13} although there are inconsistencies in the literature,¹⁴ and rises are also linked to early neurological deterioration.¹⁵ The fact that the rise in ICAM-1 levels appears attenuated by allopurinol is encouraging; levels are known to be reduced by other effective therapies.¹⁶ There may also be longer-term benefits; increased plasma concentrations of ICAM-1 are associated with both symptomatic carotid atherosclerosis¹⁷ and subcortical vascular disease¹⁸ suggesting that measures to attenuate levels could help patients with any type of cerebrovascular disease. Moreover, recent studies suggest elevated ICAM-1 is a particularly strong predictor of incident type 2 diabetes,^{19,20} whereas other evidence supports a link between elevated UA and diabetes.²¹ Thus, future studies should address whether allopurinol has potential to attenuate progression to diabetes in the poststroke period and more generally.

We excluded those with clinical infection and elevated CRP from analyses of inflammatory markers. This was decided before study completion and when the study was blinded because we anticipated that many would sustain infection. More patients had infection with marked baseline CRP elevation in the placebo group. Unsurprisingly, all exhibited marked falls in inflammatory markers as infection resolved. When these participants were included in post hoc sensitivity analyses, the differences between the groups were again in favor of a beneficial effect of allopurinol, although statistical significance was not reached.

We saw no differences in CRP and IL-6 levels among groups and no change in their levels in the poststroke period. Equally, there were no differences in ICAM-1 levels by 1 week, but levels had risen significantly in the placebo group by 6 weeks. Others have demonstrated an early rise in CRP levels, which, in one study, began to decline at 1 week.¹⁴ It is possible that we have missed an early CRP rise (and possibly a rise in IL-6) by the sampling intervals we chose. However, overall, we are reassured by the similarities among groups on all baseline measures and by the dose-response relationship we saw and feel that our results reflect a genuine effect of allopurinol treatment. We must however caution that our findings should be supported by future work to link allopurinol use to improvements in other measures of endothelial function such as

cerebrovascular reactivity or, for example, carotid intima media thickness.

In summary, our data provide the first evidence of a beneficial effect of allopurinol on surrogate markers of vascular and metabolic health after stroke and are encouraging. We have demonstrated a safe dose-dependent reduction in UA after allopurinol use together with an attenuated rise in ICAM-1, both changes that encourage further investigation of the benefits of allopurinol after stroke.

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