

Kidney International, Vol. 67 (2005), pp. 1739–1742

Hyperuricemia induces endothelial dysfunction

UDAY M. KHOSLA, SERGEY ZHARIKOV, JENNIFER L. FINCH, TAKAHIKO NAKAGAWA, CARLOS RONCAL, WEI MU, KARINA KROTOVA, EDWARD R. BLOCK, SHARMA PRABHAKAR, and RICHARD J. JOHNSON

Baylor College of Medicine, Department of Medicine, Division of Nephrology, Houston, Texas; Texas Tech University Health Science Center, Department of Medicine/Nephrology, Lubbock, Texas; and Department of Medicine, University of Florida, Gainesville, Florida

Hyperuricemia induces endothelial dysfunction.

Background. Hyperuricemia has been linked to cardiovascular and renal diseases, possibly through the generation of reactive oxygen species (ROS) and subsequent endothelial dysfunction. The enzymatic effect of xanthine oxidase is the production of ROS and uric acid. Studies have shown that inhibiting xanthine oxidase with allopurinol can reverse endothelial dysfunction. Furthermore, rat studies have shown that hyperuricemia-induced hypertension and vascular disease is at least partially reversed by the supplementation of the nitric oxide synthase (NOS) substrate, L-arginine. Therefore, we hypothesized that uric acid induces endothelial dysfunction by inhibiting nitric oxide production.

Methods. Hyperuricemia was induced in male Sprague-Dawley rats with an uricase inhibitor, oxonic acid, by gavage; control rats received vehicle. Allopurinol was placed in drinking water to block hyperuricemia. Rats were randomly divided into four groups: (1) control, (2) allopurinol only, (3) oxonic acid only, and (4) oxonic acid + allopurinol. Rats were sacrificed at 1 and 7 days, and their serum analyzed for serum uric acid and nitrites/nitrates concentrations. The effect of uric acid on nitric oxide production was also determined in bovine aortic endothelial cells.

Results. Oxonic acid induced mild hyperuricemia at both 1 and 7 days ($P < 0.05$). Allopurinol reversed the hyperuricemia at 7 days ($P < .001$). Serum nitrites and nitrates (NO_x) were reduced in hyperuricemic rats at both 1 and 7 days ($P < .001$). Allopurinol slightly reversed the decrease in NO_x at 1 day and completely at 7 days ($P < .001$). There was a direct linear correlation between serum uric acid and NO_x ($R^2 = 0.56$) and a trend toward higher systolic blood pressure in hyperuricemic rats ($P = \text{NS}$). Uric acid was also found to inhibit both basal and vascular endothelial growth factor (VEGF)-induced nitric oxide production in bovine aortic endothelial cells.

Conclusion. Hyperuricemic rats have a decrease in serum nitric oxide which is reversed by lowering uric acid levels. Soluble uric acid also impairs nitric oxide generation in cultured endothelial cells. Thus, hyperuricemia induces endothelial dysfunction; this may provide insight into a pathogenic mecha-

nism by which uric acid may induce hypertension and vascular disease.

Endothelial dysfunction, particularly impaired nitric oxide production, is a common finding in patients with cardiovascular and renal diseases and is thought to be mediated in part by reactive oxygen species (ROS) [1]. ROS can be generated by several mechanisms, one of which involves reaction of xanthine oxidase with xanthine to generate superoxide anion and uric acid. Several studies have reported that xanthine oxidase inhibitors such as allopurinol can reverse endothelial dysfunction in subjects with congestive heart failure [2, 3] or type 2 diabetes mellitus [4]. These latter studies assume that the benefit of xanthine oxidase inhibition was solely via its ability to lower ROS; however, xanthine oxidase inhibitors also lower uric acid. Indeed, there is also strong evidence linking uric acid with cardiovascular and renal disease [5]. Specifically, animals made hyperuricemic by administering an inhibitor of uricase (which happens to also block oxidant formation) develop hypertension and vascular disease, which is at least partially reversed by supplementing with L-arginine, a nitric oxide synthase (NOS) substrate [6]. A study in healthy volunteers has also shown that serum uric acid inversely fluctuates with the potent vasodilator nitric oxide during a 24-hour period [7]. Given these findings, we hypothesized that hyperuricemia may induce endothelial dysfunction by inhibiting the production of nitric oxide.

METHODS

In vivo studies

Male Sprague-Dawley rats were housed in standard conditions and fed normal diets. We induced hyperuricemia with an uricase inhibitor, oxonic acid (750 mg/kg/day), by gavage, with control rats receiving vehicle. Allopurinol was used to block hyperuricemia by placing allopurinol in the drinking water (150 mg/L). Rats were divided into four groups: (1) control, (2)

Key words: nitric oxide, uric acid, endothelial dysfunction.

Received for publication May 20, 2004

and in revised form September 8, 2004

Accepted for publication November 23, 2004

© 2005 by the International Society of Nephrology

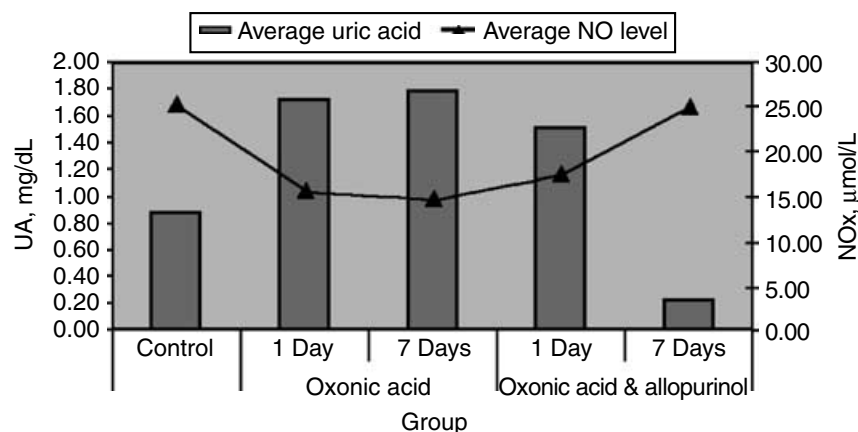


Fig. 1. The relationship of serum uric acid (UA) and serum nitrites (NO_x) at 1 and 7 days.

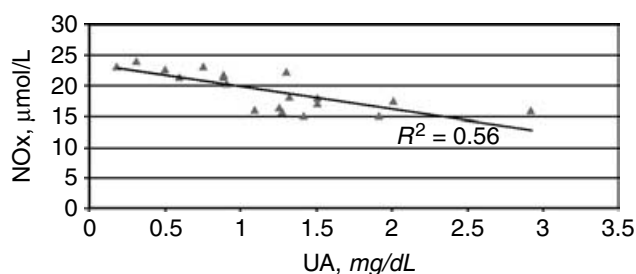


Fig. 2. Correlation of serum uric acid (UA) and serum nitrites (NO_x).

allopurinol only, (3) oxonic acid only, and (4) oxonic acid + allopurinol. Systolic blood pressure was measured using a tail-cuff sphygmomanometer. The amount of drinking water consumed and changes in body weight were noted. Rats were sacrificed at 1 and 7 days. Serum was analyzed for uric acid concentration and nitrites/nitrates (NO_x) by chemiluminescence method [8]. Statistical analysis between subgroups was performed using analysis of variance (ANOVA).

In vitro studies

Bovine aortic endothelial cells (BAEC) (passages 4 to 8) (Cambrex, East Rutherford, NJ, USA) were cultured in endothelial growth media (EBM) with Bullet Kit (Cambrex). The effect of soluble uric acid (2.5 to 7.5 mg/dL) on nitric oxide production was measured in real-time based on the fluorescence of 4,5-diaminofluorescein (DAF-2) which binds to nitric oxide [9]. Specifically, control BAEC and BAEC treated with different concentrations of uric acid (2.5 to 7.5 mg/dL for 24 hours) were first washed in Hank's balanced salt solution (HBSS) and then incubated with 5 μmol/L DAF-FM diacetate (Molecular Probes, Eugene, OR, USA) for 30 minutes at 37° C in darkness. After the incubation, BAEC were washed to remove excess probe. Fresh HBSS containing 100 μmol/L L-arginine (a substrate for nitric oxide synthesis) was added to cells, and cells were incubated for an additional 10 minutes to allow complete

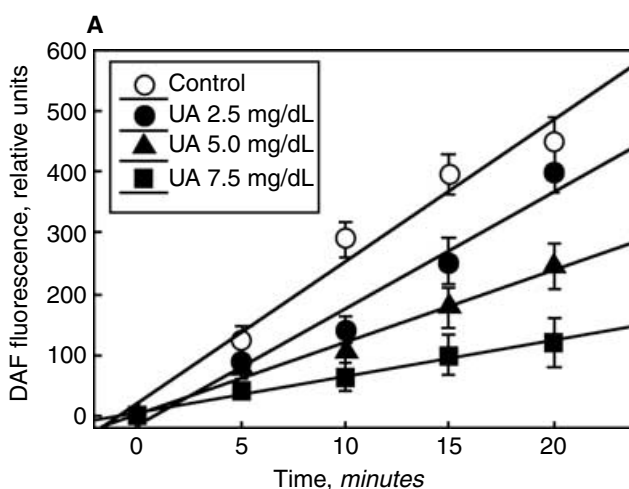


Fig. 3. Effects of uric acid (UA) on nitric oxide production by bovine aortic endothelial cells (BAEC). Cultured BAEC growing on 30 mm dishes with a glass bottom were treated with 2.5 to 7.5 mg/dL uric acid in RPMI culture media without serum for 24 hours. Visualization of nitric oxide (NOO) production using a fluorescent probe 4,5-diaminofluorescein (DAF-FM) has been done as described in the **Methods** section. (A) Intensity of DAF fluorescence inside four control cells and four cells treated with uric acid (2.5, 5.0, or 7.5 mg/dL) was quantified every 5 minutes for 20 minutes. Intensity at the 0 point time was taken as a background and all measurements were corrected by subtracting background fluorescence. Each point represents an average DAF fluorescence in one cell \pm SD. All changes in nitric oxide production in BAEC treated with 5.0 and 7.5 mg/dL uric acid were significant compared with control BAEC with $P < 0.05$ (an unpaired, two-tailed Student t test). (B) DAF fluorescence images of control (A1 and A2) BAEC and BAEC treated with 7.5 mg/dL uric acid (B1 and B2). The images of fluorescence were made just after the procedure of DAF-FM de-esterification (the 0 time point; see the **Methods** section) (A1 and B1) and after the 20-minute period of measurements (A2 and B2).

de-esterification of the intracellular diacetate. After this procedure, direct visualization of nitric oxide production with the fluorescent indicator was performed using a laser scanning confocal microscope with excitation and emission maxima at 495 and 515 nm, respectively. Intensity of fluorescence was quantified using LSM 510 (version 3.0 SP3) software for the Carl Zeiss Laser Scanning Microscope (Carl Zeiss, Inc.).

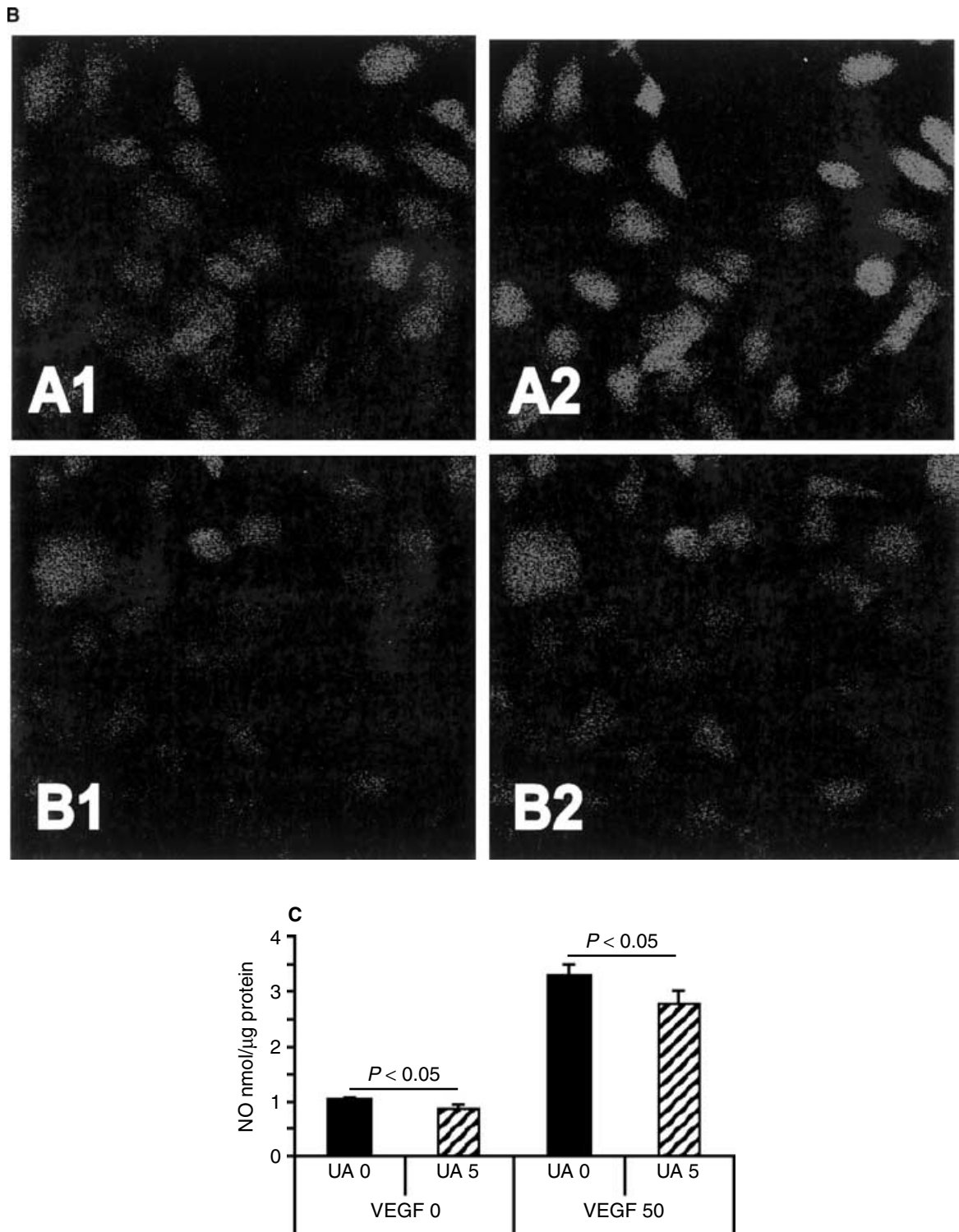


Fig. 3. (continued)

The amount of nitric oxide released into the cell media was also measured in the gas phase using a standardized Seivers NOA 280 chemiluminescence analyzer (Analytix, Durham, UK), after cells were stimulated with vas-

cular endothelial growth factor (VEGF₁₆₅) (50 ng/mL) in EBM with 5% serum for 24 hours. Results are corrected for background levels of nitric oxide present in culture medium alone, and are expressed as nanomol per

microgram (nitric oxide/total protein) and as a mean \pm SEM of three independent experiments performed in triplicate determinations.

RESULTS

There was no difference in the amount of water consumed and the change in body weight between the three groups over seven days. Oxonic acid induced a mild hyperuricemia at both 1 day (1.7 ± 0.7 vs. 0.8 ± 0.4 mg/dL in oxonic acid vs. control) ($P < 0.05$) and 7 days (1.8 ± 0.4 vs. 0.9 ± 0.7 mg/dL in oxonic acid vs. control) ($P < 0.05$). Allopurinol only had a mild and nonsignificant effect on serum uric acid concentrations at day 1 (1.52 ± 0.3 mg/dL) ($P = \text{NS}$), but effectively reversed the hyperuricemia at 7 days (0.3 ± 0.2 mg/dL) ($P < 0.001$). Serum nitrites and nitrates (NO_x) were reduced by 40% to 50% in hyperuricemic rats at both 1 day (15.6 ± 0.4 vs. 22.6 ± 1.0 $\mu\text{mol/L}$ in oxonic acid vs. control) ($P < .001$) and 7 days (14.6 ± 1.1 vs. 27.5 ± 1.3 $\mu\text{mol/L}$ in oxonic acid vs. control) ($P < .001$). This decrease in NO_x was improved slightly by allopurinol at 1 day (17.4 ± 0.8 $\mu\text{mol/L}$) ($P < .001$) and reversed completely at 7 days (25.0 ± 0.8 $\mu\text{mol/L}$) ($P < 0.001$) (Fig. 1). There was also a direct linear correlation between serum uric acid and NO_x (Fig. 2). Rats treated with allopurinol alone did not show a significant change in either serum uric acid or NO_x concentration. Rats treated with oxonic acid also showed a trend toward higher systolic blood pressure at 7 days (178 ± 18 vs. 158 ± 16 vs. 147 ± 11 mm Hg in oxonic acid vs. control vs. oxonic acid/allopurinol) ($P = \text{NS}$).

Additional studies were performed to determine if uric acid affected nitric oxide production in cultured endothelial cells. Uric acid dose-dependently inhibited nitric oxide production (Fig. 3A and B) as measured by DAF-FM fluorescence; uric acid (5 mg/dL) also blocked VEGF-induced nitric oxide release into the culture media (Fig. 3C).

CONCLUSION

Most mammals have the enzyme uricase that degrades uric acid to allantoin with the generation of oxidants. In humans, uricase is mutated resulting in higher uric acid levels. Rats administered an uricase inhibitor (oxonic acid) develop mild hyperuricemia, hypertension, and vascular disease that is mediated by activation of the renin-angiotensin system, a loss of macula densa NOS, and the development of microvascular disease [6]. In this study we also demonstrate that hyperuricemic rats have a fall in serum nitrites (a reflection of nitric oxide production) that

is reversed by allopurinol. Furthermore, there was a direct linear correlation between serum uric acid and serum nitric oxide. The induction of hyperuricemia also showed a trend toward increased systolic blood pressure. In addition, we found that uric acid also impaired both basal and VEGF-induced nitric oxide production in cultured endothelial cells. This data suggest that hyperuricemia may cause endothelial dysfunction. Interestingly, Waring et al [10] recently reported that the infusion of uric acid into humans does not impair endothelial function over a 1-hour period. However, these studies did not measure nitric oxide levels nor mention effects of *sustained* hyperuricemia on endothelial-dependent vasodilatation. These discrepancies can also be explained by differences in methods and species, suggesting the need for further studies to dissect out the complex relationship of uric acid to endothelial function and cardiovascular disease.

ACKNOWLEDGMENT

Support for these studies was provided by DK-52121, HL-68607, and DK-64233 to R. Johnson.

Reprint requests to Uday M Khosla, M.D., Baylor College of Medicine, Department of Internal Medicine, Section of Nephrology, 6550 Fannin, Suite 1273, Houston, TX 77030.
E-mail: ukhosla@tmh.tmc.edu

REFERENCES

- BERRY CE, HARE JM: Xanthine oxidoreductase and cardiovascular disease: Molecular mechanisms and pathophysiological implications. *J Physiol* 555:589–606, 2004
- FARQUHARSON CA, BUTLER R, HILL A, et al: Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation* 106:221–226, 2002
- DOEHNER W, SCHOENE N, RAUCHHAUS M, et al: Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: Results from 2 placebo-controlled studies. *Circulation* 105:2619–2624, 2002
- BUTLER R, MORRIS AD, BELCH JJ, et al: Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 35:746–751, 2000
- JOHNSON RJ, KANG DH, FEIG D, et al: Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension* 41:1183–1190, 2003
- MAZZALI M, HUGHES J, KIM YG, et al: Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension* 38:1101–1106, 2001
- KANABROCKI EL, THIRD JL, RYAN MD, et al: Circadian relationship of serum uric acid and nitric oxide. *JAMA* 283:2240–2241, 2000
- PRABHAKAR SS: Inhibition of mesangial iNOS by reduced extracellular pH is associated with uncoupling of NADPH oxidation. *Kidney Int* 61:2015–2024, 2002
- Itoh Y, MA FH, HOSHI H, et al: Determination and bioimaging method for nitric oxide in biological specimens by diaminofluorescein fluorometry. *Anal Biochem* 287:203–209, 2000
- WARING WS, ADWANI SH, BREUKELS O, et al: Hyperuricaemia does not impair cardiovascular function in healthy adults. *Heart* 90:155–159, 2004