Effect of vitamin E therapy on serum uric acid in DOCA-salt-treated rats

B Seifi, M Kadkhodaee, M Zahmatkesh

Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received: January 20, 2009
Accepted after revision: January 11, 2011

Uric acid is considered as an antioxidant in the blood. Despite its proposed protective properties, elevated plasma uric acid has been associated with hypertension in a variety of disorders. The purpose of this study was to investigate the relationship between the increase of arterial blood pressure and the changes in serum uric acid, measured during the gradual development of experimental hypertension in deoxycorticosterone (DOCA)-salt-treated rats. Blood pressure was monitored by tail-cuff method, urinary and plasma uric acid was measured by autoanalyzer during the induction of hypertension in 1-, 2-, 3- and 4-week DOCA-salt-treated Sprague–Dawley rats. Vitamin E (200 mg/kg/day/gavage) was co-administered with DOCA-salt for 4 weeks. From the first week of DOCA-salt treatment, rats exhibited marked increases in blood pressure. DOCA-salt treatment also resulted in a significant increase in serum uric acid and a significant decrease in urinary uric acid at the end of the first week. These changes in serum and urinary uric acid remained until the 4th week of DOCA-salt treatment but blood pressure continued to increase throughout the study. Vitamin E treatment increased urinary excretion of uric acid and decreased blood pressure and serum uric acid in DOCA-salt-treated rats. These data suggest that enhanced serum uric acid may be a contributing factor to the onset of hypertension in DOCA-salt-treated rats. A uricosuric effect is suggested for vitamin E in the treatment of hypertension.

Keywords: blood pressure, DOCA-salt hypertension, uric acid, vitamin E

Recent study suggests that hyperuricemia is associated with increased development of hypertension, cardiovascular diseases and chronic renal failures (2, 11, 15). It is also common in subjects with untreated hypertension (21). However, hyperuricemia not only a marker of hypertension but may also play a pathogenic role in the progression of disease (14). It has been demonstrated that uric acid induces endothelial dysfunction (21). In a recent study, it has been shown that uric acid correlates with and predicts the development of obesity, hypertension and cardiovascular disease, conditions associated with oxidative stress (22).

DOCA-salt treatment results in a low renin hypertension that is dependent on increased salt intake and DOCA treatment, and is generated in a rapid time course. The exact role of uric acid in DOCA-salt hypertension, however, remains uncertain. During tissue injury, purines are liberated and with hypoxia ATP is degraded to both adenine and xanthine, substrates for production of uric acid. In addition, hypoxia is a potent inducer of xanthine oxidase, the enzyme responsible for uric acid production (20). With the parallel increases in substrates and enzyme concentrations, uric acid production will increase. Furthermore vasospasm and loss of fluid secondary to endothelial dysfunction also stimulate renal reabsorption of uric acid (8).
In a recent study, it has been reported that greater intakes of total vitamin C were significantly associated with lower serum uric acid concentrations. These findings support a potential role of vitamin C in the prevention of hyperuricemia and gout (9). Vitamin E is a highly efficient lipid soluble antioxidant. However, the effect of vitamin E supplementation on serum uric acid levels in DOCA-salt-treated rats has not been yet studied.

Thus, in the present study we investigated the role of serum uric acid in relation to the onset of hypertension in DOCA-salt treated rats. We also tested the effect of vitamin E on serum uric acid levels in rats treated with DOCA-salt.

Methods

Experimental protocol

The study was performed on male Sprague–Dawley rats weighing between 200 and 250 g. Rats were maintained in the animal quarters under standardized conditions. All rats were maintained on a 12-h light/day cycle with 20–22°C temperature and 40–50% humidity and received standard laboratory rat chow and water ad libitum. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Tehran Medical Sciences University.

Rats were anesthetized with a combination of ketamine (50 mg/kg) and xylazin (5 mg/kg). Right unilateral nephrectomy was performed with the use of a retroperitoneal approach. After a right flank incision, the kidney was visualized. The right ureter and renal vessels were isolated and then sectioned between two ligatures. The fat and connective tissues surrounding the right kidney were removed while care was taken to avoid damaging the adjacent adrenal gland. The flank incision was closed with 3–0 silk suture materials. Postoperatively, a single injection of ampicillin sodium (22 mg/kg im) was given.

One week after unilateral nephrectomy, 64 rats were randomly divided into two groups: 1) sham-operated rats received vehicle injections and normal tap drinking water; 2) DOCA-treated rats received subcutaneous injection of deoxycorticosterone acetate (DOCA, Iran Hormone Co, Iran) at 20 mg/rat/week and provided drinking water supplemented with 1% NaCl and 0.2% KCl till the end of the experimental periods. Rats in each group were randomly divided to 4 subgroups (n=8): At seven-day intervals, urine in each subgroup was collected for the measurement of urinary uric acid. Then rats were anesthetized with ketamine/xylazine and blood was collected from the abdominal aorta. Serum was obtained to determine the concentration of blood uric acid. Serum and urine samples were stored at −80 °C until analysis.

Another group, after unilateral nephrectomy, were considered for administration of vitamin E (alpha-tocopherol, 200 mg/kg/day/gavage) in addition to DOCA-salt for 4 weeks (n=8). Serum and urine was collected for the measurement of uric acid.

Systolic blood pressure was measured in conscious rats by the tail-cuff method connected to a transducer (pneumatic transducer) using a PowerLab/4sp data acquisition system (software Chart, version 5; ADInstruments, Australia). Systolic blood pressure was determined once a week in the morning. At least three determinations were made in every session and the mean of the lowest of the three values within 5 mm Hg was taken as the SBP level.

Serum and urinary uric acid was measured by means of autoanalyzer (Integra 800, Roche, Germany) by a carbonate phosphotungstate method against standard uric acid concentrations (Sigma, St. Louis, MO).
**Statistical analysis**
Data are expressed as the mean ± SEM. Comparisons among groups were made by one-way ANOVA followed by Duncan’s test. P< 0.05 was considered statistically significant.

**Results**

**Blood pressure in different groups**
Systolic blood pressure increased significantly in DOCA-salt treated rats compared to that in the sham group during 4 weeks of treatment (p<0.05; Fig. 1a). The increase in blood pressure achieved statistical significance at the end of the first week, reaching a very profound increase at the end of the 4th week of experimental period (Fig. 1a). Figure 1b shows that vitamin E treatment significantly decreased arterial blood pressure in the DOCA-salt hypertensive rats (p< 0.05).

![Fig. 1. Time course of systolic blood pressure elevation in rats on DOCA-salt treatment (a) and systolic blood pressure in DOCA-salt + vit E at the 4th week (b).](image)
The data are presented as mean ± SEM. * p < 0.05 compared to sham-operated; # p < 0.05 compared to previous week; † p < 0.05 compared to DOCA-salt group

**Serum uric acid in different groups**
DOCA-salt treatment resulted in a significant increase in serum uric acid at the end of the first week. Serum uric acid remained elevated until the 4th week of DOCA-salt treatment (p<0.05; Fig. 2a). Figures 3a shows that vitamin E treatment resulted in a significant reduction in serum uric acid in DOCA-salt treated rats (p<0.05).

**Urinary uric acid in different groups**
DOCA-salt treatment resulted in a significant reduction in urinary uric acid at the end of the first week. Urinary uric acid remained at this low level until the 4th week of DOCA-salt treatment (p<0.05; Fig. 2b). Figures 3b shows that vitamin E treatment resulted in a significantly higher urinary uric acid in DOCA-salt treated rats (p<0.05).
Uric acid in DOCA-salt hypertension

**Fig. 2.** Time course of serum (a) and urinary uric acid (b) in rats on DOCA-salt treatment. The data are presented as mean ± SEM. * p < 0.05 compared to sham-operated.

**Fig. 3.** Serum (a) and urinary uric acid (b) in rats on DOCA-salt treatment plus vitamins E at the 4th week. The data are presented as mean ± SEM. * p < 0.05 compared to sham-operated. # p < 0.05 compared to DOCA-salt group.

**Discussion**

In this study, deoxycorticosterone (DOCA)-salt treated rats developed an elevation of blood pressure at the end of the first week, a profound increase at the end of the second week and reached severe hypertension at the end of the 4th week. Increase in serum uric acid and decrease in urinary uric acid were observed as early as the first week. Treatment with vitamin E prevented the increase in serum uric acid and elevation of blood pressure while it increased urinary uric acid in DOCA-salt treated rats.

Serum and urinary uric acid remained constant from the 1st week until 4th week of DOCA-salt treatment but blood pressure continued to increase during this study. This suggests that an elevation in serum uric acid may be more important in the onset of hypertension than in its progression. An elevation in serum uric acid has been found to predict the development of hypertension in several studies (13, 17, 24). Feig and Johnson have reported that the strongest association of uric acid with hypertension is during the onset of essential hypertension (7). An elevated serum uric acid is common in early hypertension and was present in almost 90%
of hypertensive adolescents. The novel concept that renal microvascular injury after local ischemia results in salt-dependent hypertension, provides a pathogenic link for hyperuricemia with hypertension (12). Recently, it has been suggested in a rodent model that uric acid may stimulate the development of hypertension by inducing renal afferent arteriopathy and tubulointerstitial disease (29).

This study showed that vitamin E supplementation for 4 weeks prevented the increase in serum uric acid and elevation of blood pressure in DOCA-salt treated rats. In a similar study by Tian et al., antioxidant treatment with vitamins C and E improved renal dysfunction, lessened renal injury, and decreased arterial pressure in Dahl salt-sensitive hypertension (28). Chen et al. showed that vitamin C (1000 mg/day) and vitamin E (1000 IU/day) administration reduced oxidative stress, improved vascular function and structure, and prevented the progression of hypertension in stroke-prone spontaneously hypertensive rats (5). In the present study, the reduction in blood pressure by vitamin E supplementation might be either due to decrease in serum uric acid and/or as a result of a reduction in oxygen free radical formation.

In most clinical studies, 400 IU/day or lower doses of vitamin E demonstrated no significant reduction in cardiovascular risk (10, 16). However, there is still a controversy about the optimum dose of vitamin E for the management of cardiac dysfunction. When 800 IU/day of vitamin E was used in the SPACE and CHAOS trials, significant decreases in cardiovascular risk occurred (1, 26). Previous studies have shown a beneficial effect of 200 mg/kg vitamin E to correct the overproduction of vascular superoxide anion in DOCA-salt rats. 100, 200 and 400 mg/kg of vitamin E were shown to be effective in protecting against cisplatin-induced tissue damage in rat kidneys (3, 6).

In the present study, vitamin E supplementation increased urinary uric acid and decreased serum uric acid. Mitch et al. (18) and Stein et al. (25) have demonstrated that vitamin C exerts a uricosuric effect that was shown to be beneficial. High serum uric acid levels in uncomplicated obese, insulin-resistant and hypertensive patients is mainly a consequence of impaired renal excretion (27). Nakagawa et al. (19) have shown hyperuricemia causes glomerular hypertrophy in the rat kidney. In a recent study we have shown that treatment with vitamin E for 4 weeks significantly prevented renal damage in the DOCA-salt treated rats (23). It is well known that the excretion of uric acid in the urine is by glomerular filtration, tubular reabsorption in the proximal tubule and tubular secretion at post-reabsorptive sites (4). Thus, a reduction in accumulation of uric acid in the body may suggest a better renal function by administration of vitamin E.

These data suggest that enhanced serum uric acid may be a contributing factor to the onset of hypertension in DOCA-salt-treated rats. A uricosuric effect is suggested for vitamin E in the treatment of hypertension.

Acknowledgement
This study was supported by a grant from Tehran University of Medical Sciences.

REFERENCES

Uric acid in DOCA-salt hypertension


