Tohoku J. Exp. Med., 2004, **203**, 349-352

Short Report

Maternal Plasma Hypoxanthine Levels in Nonpreeclamptic Twin Pregnancies

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SUZUKI, S. and YONEYAMA, Y. *Maternal Plasma Hypoxanthine Levels in Nonpreeclamptic Twin Pregnancies*. Tohoku J. Exp. Med., 2004, **203** (4), 349-352 —— We have observed that the elevated plasma adenosine levels are associated with hyperuricemia in nonpreeclamptic twin pregnancies. In animal models, extracellular adenosine is taken up by cells to form adenine nucleotides or is degraded to other purine metabolites such as hypoxanthine, which is further metabolized to xanthine and uric acid. In this study, we measured plasma hypoxanthine levels to evaluate the role of adenosine in hyperuricemia among women with twin pregnancies. Maternal blood samples were taken in 13 twin and 20 singleton pregnancies at 35-36 weeks' gestation. The average maternal plasma hypoxanthine level in twin pregnancies was significantly higher than that in singleton pregnancies. In addition, the plasma hypoxanthine levels have positive correlations both with plasma adenosine and serum uric acid levels. Our results support that an increased adenosine is the main factor contributing to hyperuricemia in twin pregnancies. ——— hypoxanthine; adenosine; uric acid; twin pregnancy

An association between twin pregnancy and elevated maternal serum uric acid levels has been noted (Fischer et al. 1995; Koike et al. 1997). Some possible mechanisms leading to this consequence have been discussed (Fischer et al. 1995; Koike et al. 1997). Fischer et al. (2001) reported that maternal hyperuricemia during the third trimester of nonpreeclamptic twin pregnancies is in part due to increased uric acid production. We previously observed that elevated plasma adenosine levels were associated with hyperuricemia in nonpreeclamptic twin pregnancies (Suzuki et al. 2000a). Adenosine is a precursor for the synthesis of adenosine triphosphate, which is a potent vasodilator and a metabolic regulator in the cardiovascular system. In animals, extracellular adenosine is taken up by cells to form adenine nucleotides or is degraded to other purine metabolites such as hypoxanthine, which is further metabolized to xanthine and uric acid (Becker 1993). From these

Received March 19, 2004; revision accepted for publication May 18, 2004.

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results, we hypothesized that an elevation of adenosine is the main factor contributing to hyperuricemia in twin pregnancy. Thus an elevation of hypoxanthine correlated with increased adenosine and uric acid levels should be observed. To test this hypothesis, we measured plasma hypoxanthine levels and examined the role of adenosine in hyperuricemia among women with nonpreeclamptic twin pregnancies.

Patients and Methods

We sampled maternal arterial blood (6.0 ml) in 20 women with singleton and 13 women with dichorionic twin pregnancies at 35-36 weeks' gestation at our hospitals. Patients were recruited consecutively between January 1999 and April 2004. This study was approved by the Ethics Committee of Tokyo Rinkai Hospital and Nippon Medical School, and all subjected written informed consent for participation in this study. Patients with preeclampsia, chronic hypertension, diabetes mellitus, renal disease, idiopathic thrombocytopenia, and other systemic illuness were excluded. There were no significant differences in maternal age (30±4 vs. 31±5 years old), parity (0.4±0.3 vs. 0.4±0.4) and gestational week (35.2± 0.4 vs. 35.3±0.5 weeks) at study between women with singleton and twin pregnancies.

Blood samples were obtained at 7 : 00 to 8 : 00 a.m. after an overnight fast. The first 3.0 ml of the blood sample obtained from maternal radial artery was drawn into a heparinized syringe and immediately added to an equal volume of ice-cold stop solution (9-erythro-2-(hydroxy-3-nonyl) adenine, 120 μM; dipyridamole, 20 mM; α, β methylene adenosine-5´-diphoshate, 60 mM; and ethylenediaminetraacetic acid dipotassium salt, 4.4 mM) for measurement of plasma adenosine, hypoxanthine and xanthine concentrations. The mixtures were then centrifuged at 3000 rpm for 5 minutes at 4°C. The plasma was transferred to an ultrafiltration cone (Amicon; Millipore Corp., Bedford, MD, USA) and deproteinized by centrifugation at 6000 rpm for 1 hour at 20°C. Samples of ultrafiltrate were stored at –70°C until analysis by high-performance liquid chromatography as previously reported (Suzuki et al. 2000b). Briefly, 50 μL of the ultrafiltrate was injected into a C18 column (Radial-Pac; Waters, Milford, MD, USA) and the absorbance of the eluate was monitored continuously at 254 nm for purine activity. The detection limit of adenosine was at least 10 nmol/liter, and the intra- and interassay coefficient of variation were 4.8% and 6.7%, respectively, while the detection limit of hypoxanthine was at least 10 nmol/liter, and the intra- and interassay coefficient of variation were 5.2% and 6.7%, respectively.

Other blood samples (3.0 ml) were obtained immediately to determine serum uric acid levels by the uricase-peroxidase method (Determiner L UA; Kyouwamedix, Tokyo) using an autoanalyzer (Hitachi 7350; Hitachi, Tokyo).

Data are presented as the mean ± S.D. deviation. Statistical comparisons between two groups were determined by Student's or Welch's *t*-test. Linear regression was performed by the least-squares method. $p<0.05$ was considered significant.

Results and Discussion

Fig. 1 presents plasma adenosine, plasma hypoxanthine and serum uric acid levels in singleton and twin pregnancies. The average hypoxanthine, adenosine and uric acid levels in the twin pregnancy group (4.21±1.54 μmol/liter, 0.43±0.10 μmol/liter, and 5.33±0.99 mg/100ml, respectively) were significantly higher than those in the singleton pregnancy group (2.98±1.34 μmol/liter, 0.33±0.12 μmol/ liter, and 4.53±0.86 mg/100 ml, respectively, $p<0.05$).

Fig. 2 shows relationships among plasma adenosine plasma hypoxanthine and serum uric acid levels in singleton and twin pregnancies. A positive correlation was found between hypoxanthine (μmol/liter) and adenosine levels (μmol/ liter) (plasma hypoxanthine levels=1.0+7.0×plasma adenosine levels, $r^2=0.40$, $p<0.05$), and a positive correlation was also found between serum uric acid (mg/100 ml) and plasma hypoxanthine levels

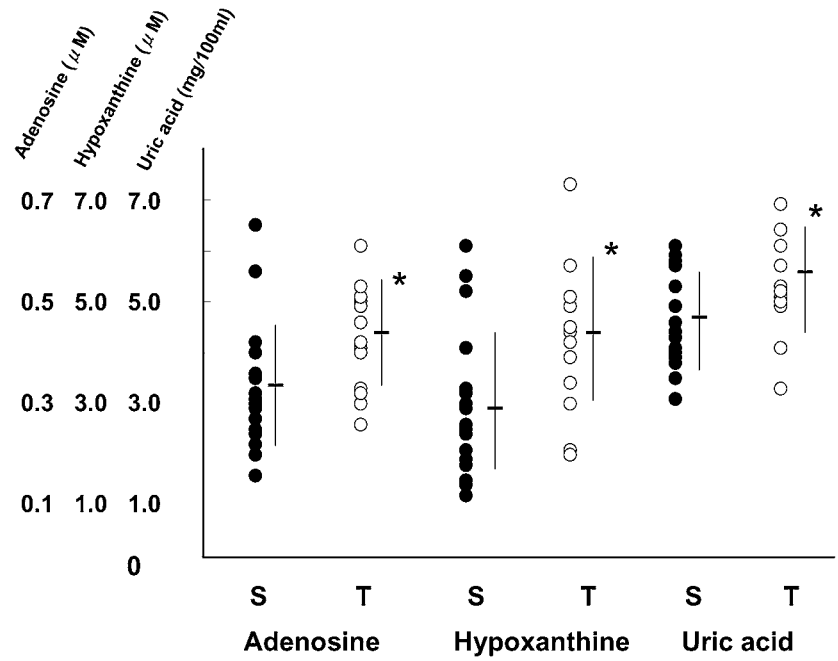

Fig. 1. The plasma adenosine, plasma hypoxanthine and serum uric acid levels in singleton and twin pregnancies. The thick vertical bar represents the mean and S.D. of determinations for each group. S, Singleton pregnanacy group; T, Twin pregnanacy group; μM, μmol/ml.
* Significantly different from the singleton pregnanacy group (p<0.05).

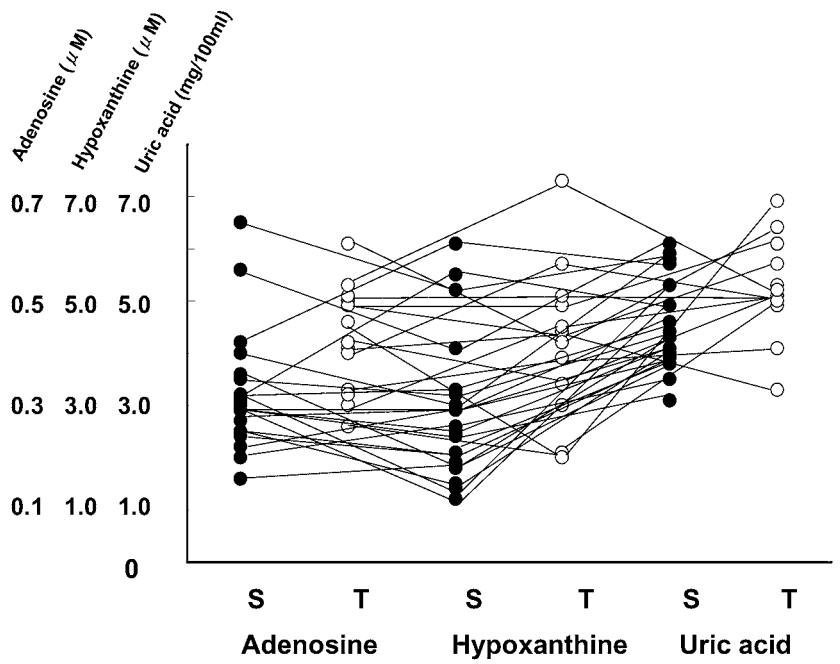

Fig. 2. The relationships among plasma adenosine, plasma hypoxanthine and serum uric acid levels in singleton and twin pregnancies.
S, Singleton pregnanacy group; T, Twin pregnanacy group; μM, μmol/ml.

(μmol/ liter) (serum uric acid=3.5+0.36×plasma hypoxanthine levels, r^2=0.36, p<0.05). In addition, a positive correlation was found between plasma adenosine (μmol/ liter) and serum uric acid (mg/100 ml) (serum uric acid levels=3.0+5.1 × plasma adenosine levels, r^2=0.53, p<0.05), as reported previously (Suzuki et al. 2000a).

The present study supports our previous report that adenosine is the main factor contributing to hyperuricemia in nonpreeclamptic twin pregnancies (Suzuki et al. 2000a). The concentration of hypoxanthine in plasma has been investigated as an indicator of the degree of tissue hypoxia (Thiringer et al. 1981), which is an important source of harmful oxygen free radicals following tissue acidosis (Fridovich 1970). During the third trimester of nonpreeclamptic twin pregnancies, the elevation of adenosine without tissue hypoxia would be reasonably predicted because low platelet count (Suzuki et al. 2000a) and/or increased pathophysiologic changes, such as cardiac output and vascular tone, are well established causes for the release of adenosine into circulating blood. In 2003, however, our previous study (Suzuki and Yoneyama 2003) indicated that vascular resistance of uterine arteries decreases in twin pregnancies through the mechanisms that are independent of adenosine. In addition, our current results may suggest the presence of a large quantity of xanthine oxidase even in nonpreeclamptic twin pregnancies.

In conclusion, the elevation of hypoxanthine levels correlated with the increased levels of adenosine and uric acid supports the concept that adenosine is the main factor contributing to hyperuricemia in nonpreeclamptic twin pregnancies. However, a further study is needed to clarify the role of purine metabolites in twin pregnancies.

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