

# Effect of Human Placenta Extract on Potassium Oxonate-Induced Elevation of Blood Uric Acid Concentration

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Although anti-inflammatory effect of human placenta extract (HPE) was observed in rheumatoid arthritis and carrageenin-induced edema, effect of HPE on the arthritis of hyperuricemia and gout patients had never been examined. Excess uric acid is regarded to be a major risk factor in gout, renal diseases, cardiovascular diseases and cerebrovascular diseases. In our experiment, in order to investigate the effect of HPE on blood uric acid levels and xanthine oxidase (XO) activity, HPE 100 mg/kg was administered to the abdominal cavity of potassium oxonate-induced hyperuricemic mice. Blood uric acid levels and XO activity were measured 3 hr after administration. Furthermore, effect of HPE on *in vitro* superoxide anion generation in xanthine-XO system was also measured. In experimental mice, oxonate-induced significant elevation of blood uric acid levels was suppressed by the HPE administration similar to the allopurinol administration. Similarly, both in HPE-treated mice and in allopurinol-treated mice, blood XO activity was significantly reduced in comparison with vehicle-treated mice and oxonate-treated mice. Furthermore, there was significant positive correlation between blood uric acid levels and XO activity ( $p < 0.01$ ). In *in vitro* experiments, xanthine concentration was increased by the addition of HPE while simultaneously reducing uric acid concentration. These results indicate that HPE contains some XO inhibitors which reduce blood uric acid levels with inhibiting uric acid generation in purine metabolism. Then, XO inhibitors of HPE could be used in the treatment of hyperuricemia and gout patients to lower blood uric acid levels.

**Key words** — placenta extract, hyperuricemia, xanthine oxidase

## INTRODUCTION

In primates, uric acid is an end-product of purine metabolism. Since only 10% of generated uric acid is excreted in urine without glomerular reabsorption, blood uric acid levels are controlled by not only its production and excretion but also enzymatic and non-enzymatic degradation thereof.<sup>1)</sup> Since uric acid efficiently scavenges free radicals and its blood levels of healthy human are physiologically maintained at approximately 0.3–0.5 mM,<sup>2)</sup> it could play as an antioxidant in several tissues to eliminate the toxic effect of endogenous hydrogen peroxide similar to catalase and glutathione peroxidase (GPx). However, excess uric acid is regarded to be a major risk factor of gout, renal diseases, cardiovascular diseases and cerebrovascular diseases.<sup>3,4)</sup> Thus, in

order to lower blood uric acid levels of hyperuricemia and gout patients, xanthine oxidase (XO) inhibitor or several uricosuric agents, such as probenecid and sulfinpyrazone *etc.*, have been frequently utilized.<sup>1)</sup> Allopurinol is only a XO inhibitor in that purpose, however it shows critical side effects while simultaneously reducing blood uric acid levels.<sup>5,6)</sup>

We recently observed an inhibitory effect of human placenta extract (HPE) to the uric acid generation in the *in vitro* xanthine-XO system. Human placenta extract prepared from the placenta of healthy pregnant female is known to have various physiological actions, including antioxidant and anti-inflammation properties. We previously reported the antioxidant activities and several antioxidants of HPE.<sup>7-10)</sup> In addition, anti-inflammatory effects of HPE were observed in rheumatoid arthritis<sup>11)</sup> and carrageenin-induced edema.<sup>12)</sup> However, there has been no observation on HPE against the arthritis of hyperuricemia and gout patients. Furthermore, it is never examined whether HPE intake influences

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blood uric acid levels. Therefore, in order to investigate the effect of HPE on hyperuricemia, blood uric acid levels and XO activity of HPE-administered mice were measured.

## MATERIALS AND METHODS

**Chemicals** — Spray-dried HPE was kindly provided from Snowden Co. Ltd. (Tokyo, Japan). Xanthine oxidase from buttermilk (EC 1.1.3.22) was acquired from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Xanthine, bovine serum albumin (BSA), allopurinol and potassium oxonate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other chemicals of the highest grade were obtained commercially.

**Animals and Treatments** — Five-week-old male ddY mice purchased from Tokyo Experimental Animal Supply Co. (Tokyo, Japan) were given MF pellet basal diet (Oriental Yeast Co., Tokyo, Japan) and tap water freely, and were used after 1 week of acclimation. Mice were housed in an air-conditioned room with temperature of  $23 \pm 1^\circ\text{C}$ , humidity of  $50 \pm 3\%$ , and a 12 hr light and dark cycle. In order to cause hyperuricemic status, potassium oxonate 300 mg/kg was quickly and carefully injected to the abdominal cavity of mice without anesthesia.<sup>13</sup> Then HPE 100 mg/kg or allopurinol 20 mg/kg was also administered to the mice 1 hr after the potassium oxonate injection.<sup>8,14</sup> All mice were sacrificed 2 hr after the administrations of HPE and allopurinol. Whole blood collected by phlebotomy was used to the measurements of blood uric acid levels, XO activity and creatinine levels. This experimental design was approved by the Animal Experimental Committee of Hoshi University and the mice were cared for in accordance with the Guidelines Concerning the Care and Use of Laboratory Animals.

**Measurement of Plasma Uric Acid Concentration** — Measurement of uric acid concentration in deproteinized plasma of mouse blood was carried out by the use of Hitachi HPLC apparatus (pump, L-6000; UV detector, L-4000; chromatointegrator, D-2500; Hitachi Co., Tokyo, Japan) according to the method of Akaike *et al.*<sup>15</sup> Detection limit of uric acid in this method was 1  $\mu\text{M}$  or more.

**Measurement of Serum Creatinine Concentration** — Serum creatinine concentration was assayed with Wako Creatinine Test (Wako Pure Chemical Industries) using a Hitachi U-2000 Spectrophotometer (Hitachi Co.).

**Measurement of Xanthine Oxidase Activity** — Xanthine oxidase activity was assayed by the method of Akaike *et al.*<sup>15</sup> Detection of uric acid formation was carried out using HPLC (Hitachi Co.). One unit of enzyme converts 1.0  $\mu\text{mol}$  of xanthine to uric acid per min.

**Measurement of Protein Concentration** — Protein concentration in each sample solution was measured by the method of Lowry *et al.*,<sup>16</sup> using BSA as the standard protein.

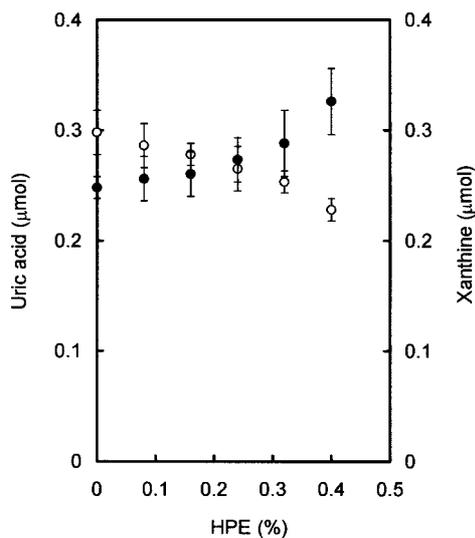
**in Vitro Uric Acid Generation** — In order to initiate uric acid generation, one unit of XO was added in 2.5 ml of sodium carbonate buffer (pH 10.2) containing 1.2 mM EDTA and 1.2 mM xanthine. After incubation at  $37^\circ\text{C}$  for 20 min, xanthine and uric acid in the deproteinized reaction mixture were measured by the method of Akaike *et al.*<sup>15</sup>

**Statistics** — Data are expressed as the mean  $\pm$  S.D. A one-way analysis of variance (ANOVA) was used to determine any significant differences ( $p < 0.05$ ) between means. When significant differences were found, Duncan's multiple-range test was used to determine the exact nature of the different.

## RESULTS AND DISCUSSION

In *in vitro* experiment, both the reduction of uric acid and the elevation of xanthine in the reaction mixture were observed when HPE was added (Fig. 1). The decreased uric acid was inversely proportional to increased xanthine. For example, in the presence of HPE 0.4%, 0.070  $\mu\text{mol}$  of uric acid decreased and 0.078  $\mu\text{mol}$  of xanthine increased in comparison with positive control (Fig. 1). These results indicate that HPE suppressed uric acid generation by inhibiting xanthine metabolism. Then HPE seems to contain some XO inhibitors, which is not investigated yet.

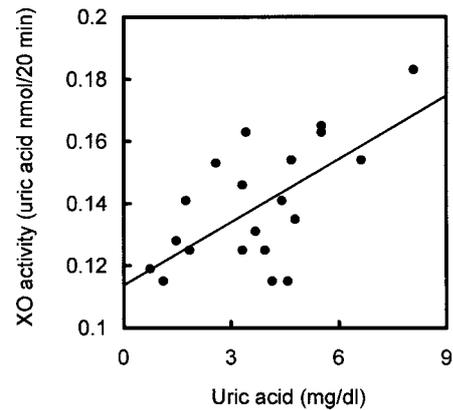
Potassium oxonate causes hyperuricemic status in rodents by inhibiting uricase activity.<sup>13,17</sup> Indeed, in our experiment, blood uric acid levels of mice increased to 189% of control in the potassium oxonate-treated group (Table 1). Potassium oxonate scarcely influences XO activity.<sup>18</sup> Indeed, in our experiment, no significant alterations of blood XO activity were observed in that group (Table 1). Therefore the elevation of blood uric acid levels seems to result from the inhibition of uric acid metabolism. Allopurinol administration significantly prevented that elevation (Table 1). Since allopurinol reduces uric acid generation by inhibiting XO activity, it has



**Fig. 1.** Effect of Human Placenta Extract on the Metabolism of Xanthine to Uric Acid by Xanthine Oxidase

Open circle and closed circle indicate uric acid and xanthine, respectively. Purines of reaction mixture were analyzed as Materials and Methods. Values are expressed as mean  $\pm$  S.D. ( $n = 3$ ).

been clinically used to mitigate the symptoms of hyperuricemia and gout patients caused by the accumulation of uric acid.<sup>14)</sup> Indeed, significant reduction of XO activity to approximately 80% of control was observed in allopurinol-treated group (Table 1). In HPE-treated mice, uricase inhibitor-induced elevation of blood uric acid levels was significantly suppressed which was similar to the allopurinol-treated mice (Table 1). Furthermore, XO activity of the HPE-treated group also significantly decreased to approximately 87% of control (Table 1). These results exhibit that HPE contains some XO inhibitors. In addition, HPE scarcely influenced uricase activity in mouse livers (data not shown). Therefore the suppressive effect of HPE on the potassium oxonate-induced elevation of blood uric acid levels of mice seems to be mainly due to the inhibition of uric acid generation by reducing XO activity simi-



**Fig. 2.** Correlation between Plasma Uric Acid Concentration and Xanthine Oxidase Activity  
Correlation coefficient is 0.640 ( $p < 0.01$ ).

lar to allopurinol. This hypothesis is also supported by the significant positive correlation between the blood uric acid levels and XO activity ( $r = 0.640$ ,  $p < 0.01$ ) (Fig. 2).

On the other hand, no significant change of blood creatinine levels was observed in all experimental groups (Table 1). Blood creatinine levels are known to elevate in several cases of kidney damage caused by the accumulated uric acid in hyperuricemia patients and in gout patients.<sup>19)</sup> Therefore our result suggests that severe kidney damage was scarcely observed in mice under these experimental conditions. An extended period seems crucial to cause kidney damage in mice through accumulated uric acid.

In hyperuricemia and gout patients, severe arthritis, arthralgia and nephrolithiasis are caused by accumulated uric acid. Furthermore, elevation of blood uric acid levels results in kidney failure, cardiovascular disease and cerebrovascular disease.<sup>4,5)</sup> In addition, elevation of uric acid levels in cortical and thalamic areas is reported to increase traumatic brain injury in rats.<sup>20)</sup> Therefore, in order to prevent

**Table 1.** Blood Uric Acid Concentration, Xanthine Oxidase Activity and Creatinine Concentration of Mice

Group	Uric acid (mg/dl)	XO (nmol/20 min/mg protein)	Creatinine (mg/dl)
Control	2.57 $\pm$ 0.84 <sup>a,c)</sup>	0.152 $\pm$ 0.011 <sup>a)</sup>	0.350 $\pm$ 0.019
Oxonate	4.87 $\pm$ 0.58 <sup>b)</sup>	0.153 $\pm$ 0.012 <sup>a)</sup>	0.332 $\pm$ 0.050
Oxonate + HPE	2.10 $\pm$ 0.37 <sup>a)</sup>	0.121 $\pm$ 0.007 <sup>b)</sup>	0.320 $\pm$ 0.042
Oxonate + allopurinol	3.10 $\pm$ 0.59 <sup>c)</sup>	0.122 $\pm$ 0.006 <sup>b)</sup>	0.314 $\pm$ 0.014

Each measurement was performed as described in Materials and Methods. Values are expressed as mean  $\pm$  S.D. ( $n = 4$ ). *a-c)* Values not sharing a common letter are significantly different at  $p < 0.05$  (ANOVA with Duncan's multiple-range test).

brain disorders, reducing blood uric acid levels of hyperuricemia and gout patients is necessary. And the inhibition of uric acid generation by XO inhibitor is one of the treatments for these patients.<sup>13)</sup> Although allopurinol is only a XO inhibitor for that purpose, it causes bone marrow depression and fulminant hepatitis *etc.* simultaneously with the decrease in blood uric acid levels.<sup>5,6)</sup> Therefore a new XO inhibitor without severe side effects is necessary for hyperuricemia and gout patients.

Our results suggest that HPE contains some XO inhibitors. Human placenta extract has been known to express no toxic effects for mammals. Then HPE containing XO inhibitor could be used for the treatment of hyperuricemia and gout patients. Since superoxide anion physiologically generates during purine metabolism, XO inhibitor is expected not only to mitigate hyperuricemic status but also to suppress oxidative stress. Indeed, several XO inhibitors are examined for utilization as antioxidants. Although we previously found the antioxidant properties and several antioxidants of HPE,<sup>7-10)</sup> HPE containing XO inhibitor seems also to be an antioxidant. Thus there should be further investigations for what components of HPE inhibit XO activity.

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