EFFECT OF *PANAX GINSENG* ON XANTHINE OXIDASE ACTIVITY IN ETHANOL-INDUCED HYPERURICEMIA

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Abstract

The influence of Panax ginseng on alcohol-induced hyperuricemia were observed.

Changes of uric acid blood levels and hepatic xanthine oxidase activities were studied by means of treating alcohol intoxication with ginseng.

It was found that a single dose (4 mg/Kg) of ginseng saponin administered intraperitoneally significantly inhibits the hepatic xanthine oxidase activities and decrease urate blood levels in ethanol-induced hyperuricemic mice.

It was also observed that there were some difference in pharmacological aspect between Panax ginseng and allopurinol which is a potent inhibitor of xanthine oxidase from any sources.

Introduction

Alteration in the biochemical reaction regulating metabolism of purines may result in change in the concentration of uric acid in the body[1-3].

A lot of consideration has been focused on drugs that lower the blood uric acid concentration, and through the studies of the action of pharmacological agents result has been accumulated concerning the nature of regulatory mechanisms[4,5].

Recently, drug-induced hyperuricemia has become a clinical problem and the study of this phenomenon also has produced some interesting information[6-8].

It is generally recognized that the ingested ethanol elevates the blood urate concentration and then aggravates pre-existing hyperuricemic diseases[9-11]. Uric acid is the main product of purine metabolism, and is synthesized from xanthine in terminal degrading processes of purine nucleotides.

In these studies, the experimental models of hyperuricemia were prepared by ethanol treatment, and the influence of ginseng was mainly observed on the xanthine oxidase activity which is concerned with uric acid formation.

Materials and Methods

Total ginseng saponin were extracted from ginseng roots according to the method of Shibata et al.[12].

Male mice weighing 20–25g were used. Animals were decapitated, the tissues were removed and blotted to remove blood. The tissues were weighed and homogenized in 0.1M potassium phosphate buffer, pH 7.5, in a glass homogenizer with teflon pestle for 1 min. The homogenate was centrifuged at 600g at 4° C for 20 min and the supernatant was centrifuged again at 105,000g at 4° C for 60 min. The supernatant was used as enzyme preparation

for the assay. The enzyme preparation was stored at -20° C and thawed immediately before the assay.

Acute ethanol intoxication was induced by 25% ethanol(1g/Kg) administration according to the method of Cohen[11].

Urate blood levels and urinary levels were determined according to the method of Caraway[13].

Uricase activity was measured by the method of Mabler[14].

Xanthine oxidase assay; The method described by Della Corte et al.[15]. Were used for the enzyme assays with slight modification. The reaction mixture had a total volume of 4 ml. The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 60 μM sodium xanthine as substrate, 500 μM of 2,8-diazahypoxanthine as uricase inhibitor[16], and 0.4 ml of the enzyme preparation. The reaction mixture was incubated at 37° C for 5 min. The reaction was stopped by the addition of 0.5 ml of 20% trichloroacetic acid solution, and the enzyme activity was measured by the increase in absorbance at 292 nm due to formation of uric acid from xanthine.

Protein was determined by the biuret[17] and lowry[18] method.

Results and Discussion

Effect of ginseng saponin on urate blood levels in ethanol-treated mice

It was found that intraperitoneal administration 1 g/Kg of 25% ethanol remarkably elevated the blood levels of urate.

The uric acid content in blood was about 2 folds higher compared with control group.

As shown in Fig. 1, a single dose of ginseng saponin markedly decreased the increment of urate blood levels in ethanol-intoxicated mice. These results suggest that ginseng has some ability to regulate uric acid blood levels in abnormally elevated condition.

Effect of ginseng saponin on the hepatic xanthine oxidase activity in ethanol-treated mice

In order to obtain data to explain the above

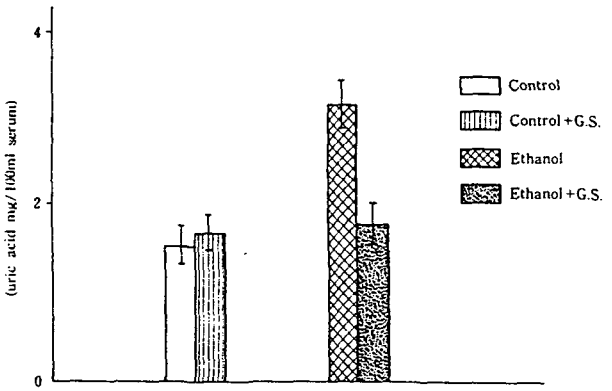

Fig. 1. Effect of ginseng saponins on the blood concentration of uric acid in mouse after alcohol treatment.

Ginseng saponin (4 mg/kg) were injected intraperitoneally to mouse 90 min before the ethanol (g/kg) treatment. The animals were sacrificed 180 min after the ginseng saponin administration. The assay procedure was described in the text. Each value represents a mean ± S.E. of four experiments. ($p < 0.01$)

ginseng effect shown in Fig. 1, we studied the influence of ginseng saponin on activity of the hepatic xanthine oxidase which catalyzes the oxidation of hypoxanthine and xanthine to uric acid. Fig. 2 shows the effect of ginseng saponin on the enzyme activity in ethanol-intoxicated mice and in control.

Administration in single dose of ginseng (4 mg/Kg i.p.) markedly decreased the enzyme

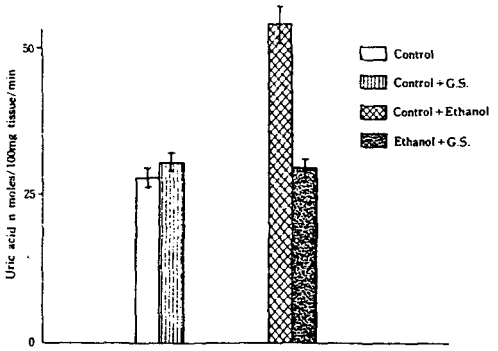

Fig. 2. Effect of ginseng saponin on xanthine oxidase activity in liver supernatant fraction in ethanol-treated mouse.

Ginseng saponin (4 mg/kg) were injected intraperitoneally to mouse 90 min before the ethanol (g/kg) treatment. The animals were sacrificed 180 min after ginseng saponin administration. The assay procedure was in the text. Each value represents a mean ± S.E. of five experiments. ($p < 0.01$)

activity to the control levels in experimentally induced hyperuricemia by ethanol treatment.

The results indicate that the ginseng might be controlled the urate concentration in blood by inhibition of the activity of xanthine oxidase which is concerned with the production of uric acid in the body.

Effect of ginseng saponin on uricase activity in ethanol-treated mice

In most mammals, urate is absorbed by the renal tububles and converted by uricase, chiefly in the liver, to more soluble allantoin which is excreted in the urine.

Increment of uricase activity in the liver also reduce the uric acid blood levels by degradation of urate.

Therefore, it was observed that the influence of ginseng on the activity of uricase in ethanol-intoxicated mice, and the results are shown in Fig. 3. These results indicate that ginseng could not alter the uricase activity in alcohol-induced hyperuricemic mice.

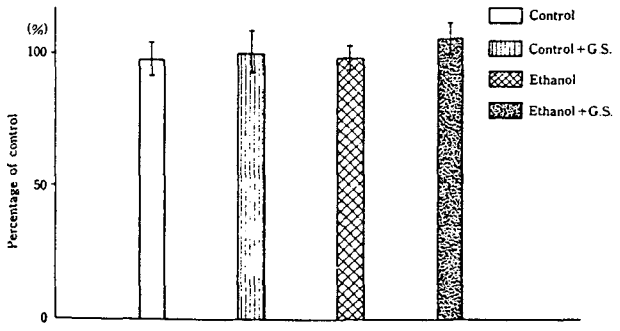

Fig. 3. Effect of ginseng saponin on the hepatic uricase activity in mouse after ethanol treatment.

Ginseng saponin (4 mg/Kg) were injected intraperitoneally to mouse 90 min before the ethanol (g/Kg) treatment. The animals were sacrificed 180 min after the ginseng saponin administration. Each value represents a mean ± S.E. of four experiments.

On the basis of these experimental results, it would be concluded that the effect of ginseng on the uric acid regulation does not appear through the change of uricase activity.

Comparison of the specificities of ginseng and allopurinol on xanthine oxidase inhibition

Ginseng saponin administered intraperitoneally to ethanol intoxicated mice in a dose of 4 mg/Kg showed some difference of action from that of allopurinol on the xanthine oxidase activity. Allopurinol is widely used for the clinical treatment of hyperuricemia such as gout by inhibition of the oxidation of hypoxanthine and xanthine to uric acid[19-21].

As shown in Fig. 4, allopurinol strongly inhibited the enzyme activity in control as in ethanol-treated groups, whereas ginseng did not affect the enzyme activity in control.

Activities of the enzymes which were prepared from intestine and kidney are not affected by ginseng treatment in both control and ethanol-intoxicated mice as shown in Fig. 5.

Thus it seems that there is a marked difference in the action mechanism of inhibition on the xanthine oxidase activity between ginseng and allopurinol.

Effect of ginseng saponin on xanthine oxidase activity in vitro

It is well known that saponin groups decrease

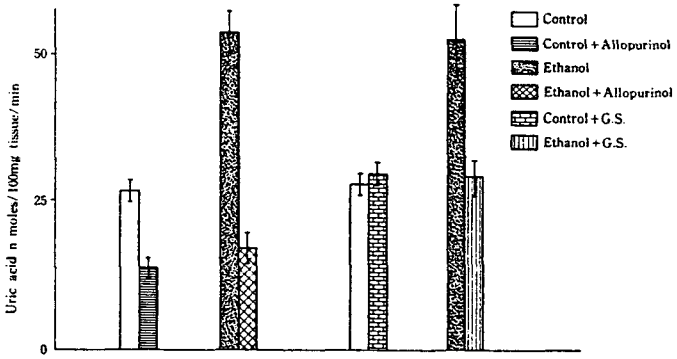

Fig. 4. A comparison of the specificities of ginseng saponin and allopurinol on the hepatic xanthine oxidase activity in ethanol-treated mouse.

Ginseng saponin (4 mg/Kg) and allopurinol (3 mg/Kg) were injected intraperitoneally to mouse 90 min before the ethanol (g/Kg) treatment. The animals were sacrificed 180 min after the ginseng saponin and allopurinol administration. The assay procedure was in the text. Each value represents a mean ± S.E. of five experiments. ($p < 0.01$)

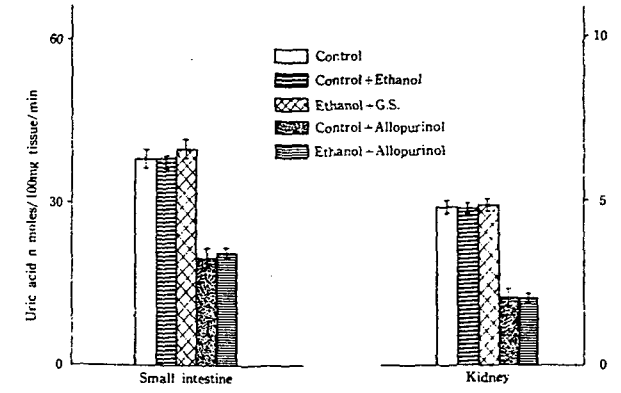

Fig. 5. Effect of ginseng saponins on the xanthine oxidase activity in small intestine and kidney.

Ginseng saponin (4 mg/Kg) and allopurinol (3 mg/Kg) were injected intraperitoneally to mouse 90 min before the ethanol (g/Kg) treatment. The animals were sacrificed 180 min after the ginseng saponin and allopurinol administration. The assay procedure was in the text. Each value represents a mean ± S.E. of five experiments. ($p < 0.01$)

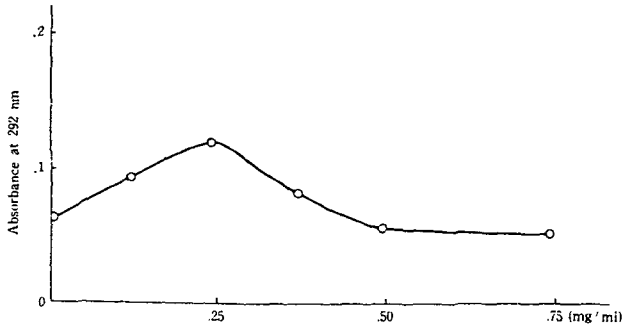

Fig. 6. Changes on the hepatic xanthine oxidase activity in various concentration of ginseng saponin in vitro.

The reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.5, sodium xanthine 6×10^{-5} M, 2,8-diazahypoxanthine 5×10^{-4} M, various concentration of ginseng saponins, and 0.4 ml of enzyme preparation. The assay procedure was in the text.

the surface tension of water. It can be readily supposed that the surfactant effect of ginseng would be able to change the xanthine oxidase activity. Therefore, it was observed that the effect of ginseng on the enzyme activity when ginseng saponin was directly added at the reaction mixture, and the rusults are shown in Fig. 6.

It is found that the ginseng saponins gradually increase the enzyme activity in accordance with the content in low concentration, whereas in high concentration remarkably inhibited the enzyme activity in vitro test.

Recently, the chemical structure and pharmacological activity of ginseng have been widely studied, and it has been reported that ginseng saponin regulate various biological reactions in the liver[22, 23].

Brekhman[24] reported that ginseng has adaptogenic activity and that the effective components of ginseng are glycosides of dammarane series.

The data presented here show that ginseng saponin has some ability to regulate plasma uric acid levels in abnormally enhanced condition. From the above results, it seems likely that the effect of ginseng might be brought, at least, partly by their inhibitory action on the hapatic xanthine oxidase.

It is very interesting that the ginseng effect was shown in abnormal state but not in control groups.

In the present work, it is also found that ginseng has selective action compared with allopurinol which is strong xanthine oxidase inhibitor as widely used in treatment of hyperuricemic disease[19, 21].

These results indicate that ginseng has some special activity to control the hyperuricemia induced by ethanol treatment. Although the action mechanism of ginseng on the regulation of uric acid blood levels is not clear, these results suggest that ginseng is a drug greatly worthy of careful study.

Chairman: Now the time is open to discussion.

Yamamoto: Thank you very much for very unique and interesting data. Judging from the ginseng effect on lipid and sugar metabolism, how about the ginseng effect on PRPP synthesis or PRPP levels.

Huh: I did not observe the effect of ginseng on RPP synthesis.

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