Biological Activities of Non-saponin Compounds Isolated from Korean Red Ginseng

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Abstract □ We have been isolating various physiologically active substances from non-saponin fraction of Korean Red Ginseng. These are adenosine, pyro-glutamic acid, dencichine and acidic polysaccharide. Adenosine and pyro-glutamic acid are known to inhibit epinephrine-induced lipolysis in fat cells and stimulate the insulin-mediated lipogenesis. In addition to these actions, adenosine was found to inhibit both norepinephrine- and histamine-induced aorta constriction, and pyro-glutamic acid inhibits angiotensin-converting enzyme. Dencichine stimulated histamine-induced aorta constriction. Finally, acidic polysaccharide was found to inhibit both lipolytic and anorexigenic actions of Toxohormone-L.

Based on these experimental results, I presented a brief review on these compounds isolated from non-saponin fraction of Korea Red Ginseng.

Keywords□ *Panax ginseng*, Korean red ginseng, adenosine, pyroglutamic acid, dencichine, acidic polysaccharide, lipolysis, lipogenesis, angiotensin-converting enzyme, toxohormone-L.

Introduction

Korean Red Ginseng is a medicinal plant long used in the treatment of various pathological states including general complaints such as head ache, shoulder ache, chilly constitution, anorexia and diabetes.

During the past two decades, physiological and biochemical approaches to elucidate the mechanism of ginseng effect on the animal body have been intensively done. Most of thest studies on ginseng were concerned mainly with ginseng saponin fractions[1-3]. However, we have isolated various physiologically active substances from non-saponin fraction of Korean Red Ginseng[4-6]. These are adenosine, pyro-glutamic acid and acidic polysaccharide.

Recently, we isolated another nitrogenous compound from non-saponin fraction of Korean Red Ginseng. This nitrogenous compound was found to be dencichine.

In the present communication, I would like to present a brief review on these compounds isolated from non-saponin fraction of Korean Red Ginseng.

Materials and Methods

Materials

Collagenase (cell diverse type) and bovine serum albumin were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Norepinephrine and histamine were obtained from Sankyo Co. Ltd. and Wako Pure Chemical Industries Ltd., respectively. D-[^{14}C(U)-glucose (2.2 mCi/m mol) was purchased from New England Nuclear.

Animals

Young male Wistar King rats weighing 160 to

200 g and male guinea weighing 300 to 350 g were given standard laboratory diet and water *ad lib.*

Panax ginseng

Red ginseng powder (*Panax ginseng* C.A. Meyer) was kindly provided by Nikkan Korai Ninjin Co. Ltd., (Kobe Japan) and Korea Ginseng and Tobacco Research Institute.

Measurement of aorta constriction

A descending thoracic aorta was excised from male guinea pig and trimmed free of adhering fat and connective tissue. A transverse strip was made by cutting across a ring. The strip was mounted for isometric recording of tension in oxygenated Tyrode solution. The solution was kept at 32 °C and bubbld with air. The mechanical response of the strip was recorded by means of an isotonic transducer (Nihon Koden SB-IT). The strip was equilibrated for 60 min under 1 g resting tension and applied with norepinephrine (10 μg/m*l*) or histamine (10 μg/m*l*) 3 times at intervals of 15 min. Thereafter, the strip was repeatedly applied with norepinephrine (0.1 μg/m*l*) or histamine (0.1 μg/m*l*) at intervals of 10 min until the contractile response of the strip attained a constant level. In the following experiments, a sample solution was introduced 1 min before application of norepinephrine or histamine.

Analysis of data

Student's t-test was used to determine the significance of difference.

Results and Discusson

Isolated rat adipocytes are well known to possess opposite pathways of lipid metabolism: lipolysis and lipogenesis. Both of the metabolism respond to various biologically active susbtances such as catecholamines and insulin. The formers stimulate lipolysis and the latter accelerates lipogenesis. Previously, we reported that non-saponin fraction of Korean Red Ginseng contains adenosine[4] and pyro-glutamic acid[5] which inhibits epinephrine-induced lipolysis and stimulated insulin-mediated lipogenesis from glucose.

Based on these results, we suggested to call these substances (adenosine and pyro-glutamic acid) "selective modulators".

Recently, we found that these substances possessed other physiological activities than those as selective modulators. The details are described in the following sections.

Effect of adenosine on aorta constriction

Adenosine was found to inhibit both norepinephrine- and histamine-induced constriction of aorta as shown in Fig. 1. In addition to adenosine, ATP, ADP and AMP also reduced both norepinephrine- and histamine-induced aorta constriction. On the other hand, IMP inhibited histamine-induced aorta constriction but not the aorta constriction induced by norepinephrine. Furthermore, GMP failed to inhibit the aorta constrictions induced by these hormones.

Anyway, above results shows that adenosine in Korean Red Ginseng possesses inhibitory activities toward aorta constrictions induced by norepinephrine and histamine in addition to the activities as a selective modulator. Therefore, it seems likely that adenosine accelerates blood circulation, expecially its peripheral circulation and improves pathological states such as shoulder ache, chilly constitution and others possibly induced by disturbance of the peripheral circulation.

Effect of pyro-glutamic acid on angiotensin converting enzyme

Korean Red Ginseng contains about 0.3% pyro-glutamic acid in its powder. Pyro-glutamic acid was found to possess an inhibitory activity toward angiotensin converting enzyme (ACE) in addition to the activity as a selective modulator. ACE was isolated from rat lung by the methods of Takada *et a.*[7]. A mixture of 2.5 mM Hip-His-Leu (0.15 m*l*) and ACE solution (0.1 m*l*; ACE activity; 2.64 units/mg protein, 1 unit: 1 μ·moles/hippuric acid/min/m*l* reaction mixture) were incubated with or without indicated amounts of test compounds at 37 °C for 30 min in a final volume of 0.35 m*l*. The reaction was

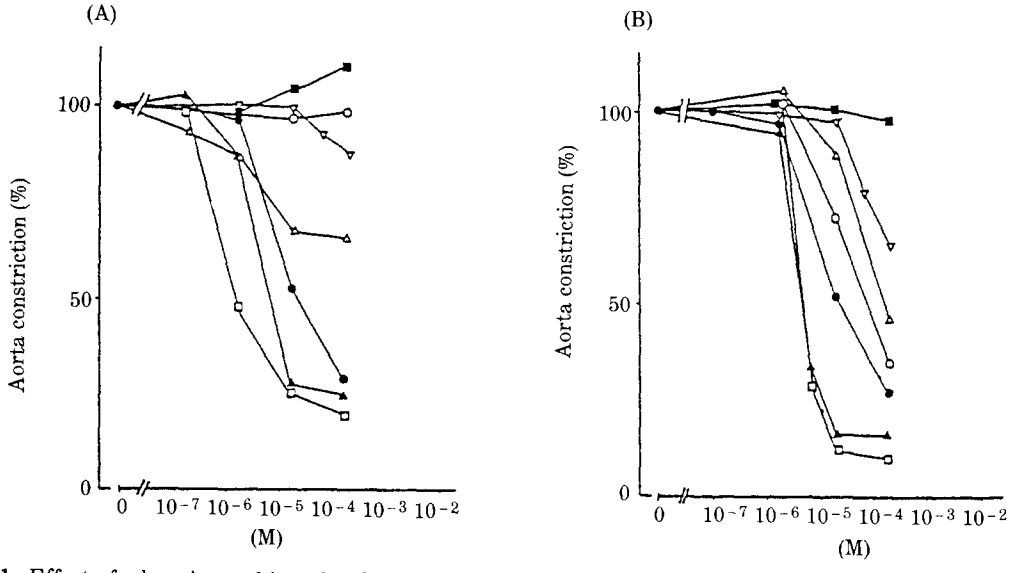

Fig. 1. Effect of adenosine and its related compounds on aorta constriction.
(A): Norepinephrine (0.59μM)-induced aorta constriction was defined as 100%.
(B): Histamine (0.54μM)-induced aorta constriction was defined as 100%.

Table 1. Effect of pyro-glutamic acid on angiotensin converting enzyme. The procedure was as described in the text.

Pyro-glutamic acid in the reaction mixture	Optical density at 228 nm	Percent inhibition (%)
None	0.497	0
1 μg	0.384	23
10 μg	0.346	30
100 μg	0.328	34
1 mg	0.173	65
10 mg	0.002	100

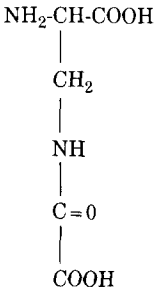

Fig. 2. Chemical structure of dencichine, β-N-oxalo-L-α, β-diaminopropionic acid.

stopped by adding 1 N HCl (0.25 m*l*) and the mixture was extracted with ethyl acetate (2.0 m*l*). The ethyl acetate phase (1.0 m*l*) was evaporated, and the residue was dissolved in water (2.0 m*l*). And then, the free hippuric acid was determined by ultraviolet (UV) absorption at 280 nm for detection. Pyro-glutamic acid clearly inhibited ACE activity at the dose of 1 μg per 0.35 m*l* of the reaction mixture as shown in Table 1.

In 1988, Kuwashima *et al.* reported that oral administration of Korean Red Ginseng to 19 hypertensive patients caused significant reduction of systolic blood pressure[8]. It seems likely that the present experimental result on pyro-glutamic acid affords a theoretical basis toward the above clinical finding.

Detection of dencichine in Korean Red Ginseng

Dencichine, β-N-oxalo-L-α, β-diaminopropionic acid, is known to be an antihemorrhagic principle (Fig. 2).

Yinan Zheng (Jilin Agricultural University, Changchun, China) examined the contents of dencichine in various ginseng preparations and found that American ginseng, Chinese ginseng and Sanchiginseng contain dencichine at the rates of 0.31%,

0.50% and 0.90%, respectively (Personal communication). Thereafter, he has been trying to estimate dencichine content of Korean Red Ginseng and examines its biological actions in our laboratory.

A hundred g of Korean Red Ginseng powder was extracted with 1 *l* of water at room temperature for 24 h. The water extract was concentrated under reduced pressure. The resulting concentrated material was extracted with butanol saturated with water to remove saponin fractions. The water soluble portion was then subjected to Sephadex LH 20, CM-Sephadex C-25 and MONO-Q column chromatographies, successively. We obtained 30 mg of dencichine from 100 g of Korean Red Ginseng.

With this dencichine, we first examined the effect of the compound on aggregation of human blood platelets. Although dencichine is supposed to be an antihemorrhagic principle, this compound did not exert any effect on the platelet aggregation induced by norepinephrine, ADP, thrombin and collagen at its concentration of 200 μg/m*l*.

However, we found that dencichine accelerated histamine-induced constriction of guinea pig aorta but not the action of norepinephrine as shown in Table 2.

Therefore, there is a possibility that dencichine exerts its antihemorrhagic action through its enhancing effect on constriction of blood vessels induced by histamine.

Table 2. Effect of dencichine on histamine-induced constriction of guinea pig aorta. Histamine (0.54μM)-or norepinephine (0.59μM)-induced aorta constriction was defined as 100%.

Agonists	Dencichine	% control
Histamine (0.54 μM)	0	100
	1	122
	10	148
	50	174
	100	191
Norepinephrine (0.59 μM)	0	100
	100	102

Biological activities of acidic polysaccharide from Korean Red Ginseng

Depletion of fat stores has been observed during progressive weight loss in patients with various neoplastic diseases. This depletion of body fat during growth of neoplasms is associated with increase in the plasma level of free fatty acids.

We found that the ascites fluid from sarcoma 180-bearing mice and patients with hepatoma or malignant ovarian tumor, and the pleural fluid from patients with malignant lymphoma elicited fatty acid release from slices of rat adipose tissue *in vitro*[9]. A lipolytic factor, named "Toxohormone-L", was purified from the ascites fluid of sarcoma 180-bearing mice and of patients with hepatoma.

Injection of Toxohormone-L into the lateral ventricle of rats significantly suppressed their food and water intakes. Therefore, Toxohormone-L has two actions: lipolytic and anorexigenic, which may cause reduction of body fat in cancer patients.

In previous studies, we reported that ginsenoside Rb_2 in Korean Red Ginseng inhibited both lipolytic and anorexigenic actions of Toxohormone-L[4,10].

In the course of this experiment, we found that Korean Red Ginseng contained another inhibitory substance toward Toxohormone-L than ginsenoside Rb_2. The inhibitory substance was found to be an acidic polysaccharide which was consisted of a pectin-like α-1,4-polygalacturonan backbone with some acetoxy groups[6].

The acidic polysaccharide inhibited Toxohormone-L-induced lipolysis in rat adipocytes. Furthermore, it inhibited anorexigenic action of Toxohormone-L. As shown in Fig. 3, the acidic polysaccharide did not affect food intake of rats, whereas it attenuated anorexigenic action of Toxohormone-L which was injected into third ventricle in the brain at a dose of 50 μg per rat.

Activity of the acidic polysaccharide was assayed by measuring the inhibitory effect of the ginsenoside-free fraction on Toxohormone-L-induced lipolysis and defining 10% inhibitor as 1 unit. The activity in large roots of Korean Red Ginseng (average diameter; 2 cm) was 3847 units/g Red Ginseng powder, whereas in small roots (average diameter;

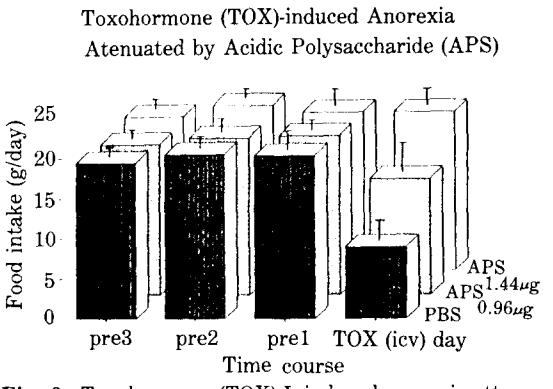

Fig. 3. Toxohormone (TOX)-L-induced anorexia attenuated by acidic polysaccharide (APS). The procedure is as described in the text.

0.5 cm) it was 1387 units/g Red Ginseng powder. Thus, large roots contain more of the acidic polysaccharide than small ones.

From ancient times, large ginseng roots have been thought to be far more effective than small ones. However, the content of ginsenosides which are believed to be main principles of pharmacological actions of ginseng, is greater in small ginseng roots than in large ones. Thus there is a contradiction between the ginsenoside content of ginseng roots and their appreciated value. In contrast to ginsenosides, the acidic polysaccharide was found more in large roots than in small ones.

The result suggests that pharmacological actions of ginseng roots may be derived from its non-saponin fractions as well as from ginsenosides. Experiments are now in progress to certify this possibility.

Acknowledgement

This work was supported by Medical Society for Red Ginseng Research.

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