STUDIES ON THE EFFECTS OF GINSENG COMPONENTS ON DIABETES MELLITUS

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Abstract

Red ginseng powder was administered at a dose of 2.7 g per day for 3 months to 21 diabetic patients who were under the treatment with insulin. It was found that the ginseng powder was effective to 12 patients and ineffective to 9 patients. Based on these clinical results, experiments were carried out to elucidate factors which concerned with improvement of pathological conditions of diabetes mellitus. In the previous symposium, we reported that red ginseng powder contained an anti-lipolytic peptide, or an insulin-like peptide. In the course of purification of the insulin-like peptide in the ginseng, we found another fraction which possessed anti-lipolytic activity. The anti-lipolytic factor of the fraction was purified by gel filtration on Bio Gel P-2 column and Dowex 50W × 4 column chromatography. The character of the finally purified material was examined by thin-layer chromatography, high-speed liquid chromatography and mass spectrometry. With these examinations, the active principle was indentified to be adenosine. Pharmacological significance of these insulin-like substances, the peptide and adenosine, was discussed.

Introduction

From ancient times, the root of Panax ginseng has been used for treatments of various diseases including diabetes mellitus, as an important folk drug in the Asian countries such as china, Korea and Japan. A large number of pharmacological studies on Panax ginseng root have been done and various effects were shown. Petkov[1] reported that oral administration of the aqueous alcoholic extract of ginseng root caused a decrease in blood sugar of rabbits, and Takeuchi[2] observed that ginseng saponins reduced the adrenaline-induced hyperglycemia. These results suggest that ginseng root contains some insulin-like substance. We reported at the 2nd international ginseng symposium that water extract of red ginseng strongly inhibited adrenaline-induced lipolysis in isolated fat cells of rat epididymal adipose tissue[3,4]. We purified the antilipolytic substance from the water extract, identified it to be an acidic peptide and called it "insulin-like peptide". It is well known that insulin inhibits adrenaline-induced lipolysis. In the course of this experiment, we found another anti-lipolytic substance in the water extract of red ginseng.

The present communication describes

purification and characterization of these insulin-like substances and clinical examinations of red ginseng powder on diabetic patients.

Materials and Methods

Animals

Young male Wistar King strain rats, weighing 160 to 200 g, were given standard laboratory diet and water ad lib. They were sacrificed by a blow on the head and their epidiymal adipose tissues were quickly removed.

Panax ginseng

Red ginseng powder (*Panax ginseng* C. A. Mayer) was kindly given by Nikkan Korai Ninjin Co. Ltd., Kobe, Japan.

Measurement of anti-lipolytic activity

Epididymal adipose tissue slices (100 mg) were incubated for 2 h at 37°C in Krebs-Ringer phosphate buffer containing 21% bovine albumin, adrenaline 0.25 μg and various test samples in a final volume of 1.2 ml. After incubation, 5 ml of Dole's extraction mixture was added and free fatty acids released were estimated by the method of Dole[5]. Ten per cent inhibition of adrenaline-induced lipolytic activity by test samples was defined as one unit.

Protein determination

Protein was estimated by the method of Lowry[6].

Column chromatography

Gel filtration was carried out on Bio Gel P-2 column. Elution was done with water. Dowex 50W × 4, 200–400 mesh in H^+ form, was used. Elution was performed by a linear gradient from 0 to 2 N HCl.

Thin layer chromatography

Thin layer chromatography was carried out on Silicagel 60 with the mixture of n-butanol: acetic acid: water (3:1:1) as developing solvent.

High-speed liquid chromatography

High-speed liquid chromatography was done using μC_{18} Waters column with the mixture of methanol: H_2O (15:85) as solvent.

Preparation of trimethylsilyl derivatives

After lyophilizing, 50–100 μg of dried sample were heated with 100 μl of N, O-bis (trimethylsilyl) trifluoroacetamide and 1.5 μl of trimethychlorosilane at 80°C for 1 hr in a sealed ample on glycerol bath. Adenosine was also trimethylsilylated by the same procedure.

Instrumentation

Gas chromatographic with mass spectrometric analysis was carried out with helium as the carrier gas using a JMS-D300 Mass Spectrometer. The column was packed with 80–100 mesh AW-DMCS chromosorb W coated with 2% OV-17 silicone.

Results and Discussion

Clinical studies on red ginseng powder

Red ginseng powder was orally administered at a dose of 2.7 g per day for 3 months to 21 diabetic patients who were under the treatment of insulin. After administration of the ginseng powder for 3 months, following clinical results were found: Blood glucose control could be achieved without administrating insulin in 3 patients. Blood glucose could be controlled by reduced amount of insulin in 5 patients. Diabetic retinoangiopathy was improved in 2 patients. Blood pressure was normalized in 1 patient and shoulder ache was improved in 1 patient.

Separation of insulin-like substances from red ginseng

Based on these clinical results, experiments were designed to isolate insulin-like substances from red ginseng. In our previous report[4], it was

demonstrated that water extract of Panax ginseng contained an insulin-like peptide, or an antilipolytic peptide. Although the anti-lipolytic peptide was isolated in a purified form, the yield was low (0.4%) and the pruification procedure were complicated. Therefore, we tried to improve the purification procedure. Panax ginseng powder was extracted with 9 fold of water at 4°C for 24 hr. After centrifugation, the water extract was concentrated, and dialyzed against water at 4°C for 24 hr. The procedure was repeated twice. The combined outer dialysate possessed about 85% of antilipolytic activity of the extract (Table 1). The outer dialysate was

Table 1. Purification of anti-lipolytic substance

Procedure	Total activity (units × 10^3)	Recovery (%)
crude extract	1399	100.0
inner dialysate	279	19.9
outer dialysate	1184	84.6
Bio Gel P-2 column chromatography		
fraction I	0	0
II	359	25.7
III	100	7.2
IV	256	18.9
V	0	0
VI	0	0
VII	0	0
2nd Bio Gel P-2 column chromatography		
fraction I	0	0
II	0	0
III	0	0
IV	0	0
V	189	13.5
VI	0	0
Dowex 50W × 4 column chromatography		
main peak	192	13.7

concentrated and applied on Bio Gel P-2 column.

As shown in Fig. I, anti-lipolytic activities were eluted mainly in fraction II and IV. Fraction II was identified to be a peptide fraction, while fraction IV was characterized by high absorption at 260 nm. Fraction IV was re-chromatographied on Bio Gel P-2 column as shown in Fig. 2. The active fraction was collected and then applied on a Dowex 50W × 4 column. Elution was carried out with linear gradient from 0 to 2 N HCl.

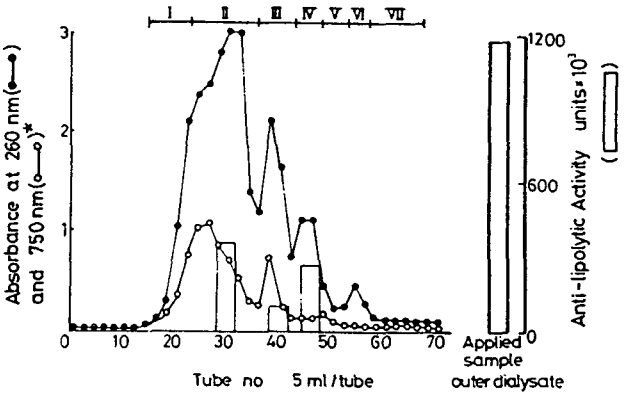

Fig. 1. Gel filtration of outer dialysate on Bio Gel P-2 column. column size: 2.2 × 43 cm. Elution was carried out with water.
* Protein was estimated by Lowry's method.

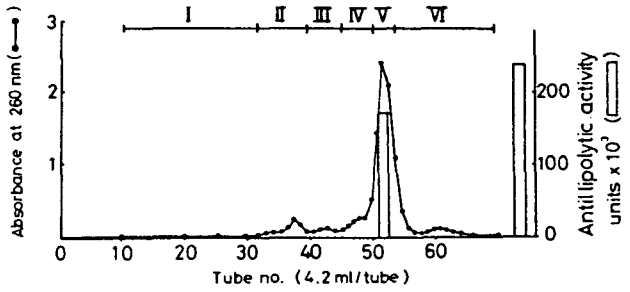

Fig. 2. Rechromatography of Bio Gel P-2 fraction IV on a Bio Gel P-2 column
Column size 2.2 × 43 cm
Elution was carried out with water.

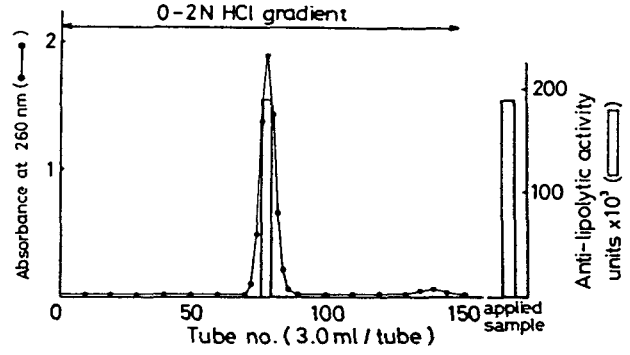

Fig. 3. Ion exchange chromatography on a Dowex 50 W × 4 column
column size: 1.0 × 15 cm

As shown in Fig. 3, all of the anti-lipolytic activity was eluted at the main peak. This fraction also possessed high absorption at 260 nm (Fig. 4). The absorption ratio (A 280 nm/A 260 nm) was examined and that of the main peak was found to be 0.186 which was the same value as that of adenosine. The active fraction was applied

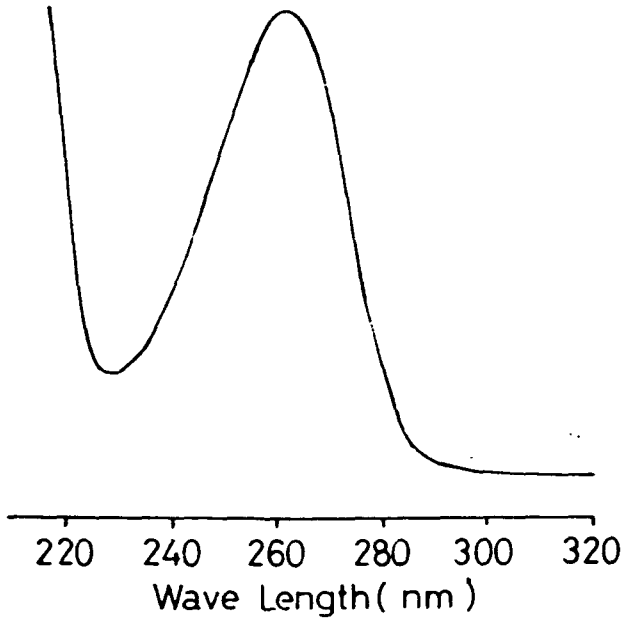

Fig. 4. Ultraviolet absorption spectrum of Dowex 50 W × 4 eluate (main peak)

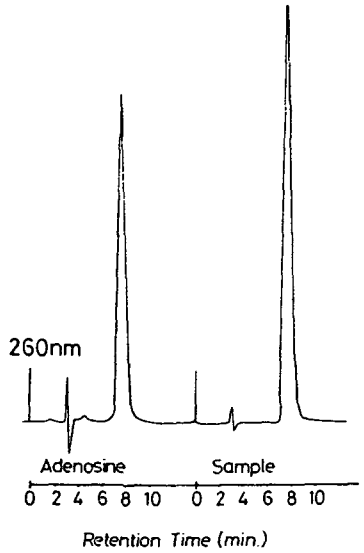

Fig. 5. High-speed liquid chromatogram of Dowex 50 W × 4 column eluate (main peak)

on Sillicagel 60 thin layer chromatography. Rf value of the fraction was found to be the same as that of adenosine. Then, the fraction was applied to high-speed liquid chromatography.

As shown in Fig. 5, the fraction showed the same retention time as that of adenosine. Next the fraction was analyzed by mass spectrometry. The mass spectra of TMS-derivatives of the fraction and adenosine were compared. It was

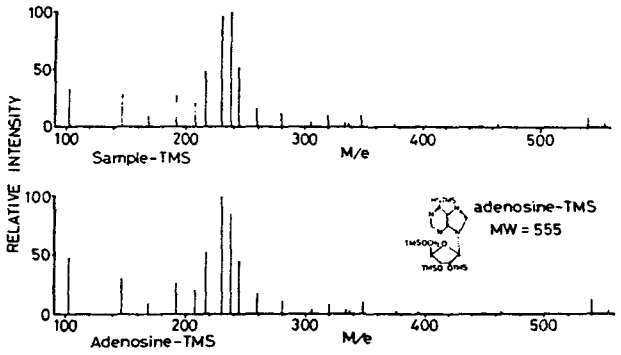

Fig. 6. Mass spectra of the trimethylsilyl derivatives of adenosine and Dowex 50W × 4 column eluate (main peak)

found that the TMS-derivative of the fraction showed the same intensity of each fragmented ion that of the TMS-derivative of adenosine (Fig. 6). Based on these examinations, the active principle was identified to be adenosine.

Physiological significance of insulin-like substances in red ginseng

We have discovered two kinds of insulin-like substances in red ginseng, an acidic peptide[4] and adenosine. These substances showed anti-lipolytic activity to ACTH and growth hormone-induced lipolysis in addition to adrenaline-mediated lipolysis. Furthermore, both substances

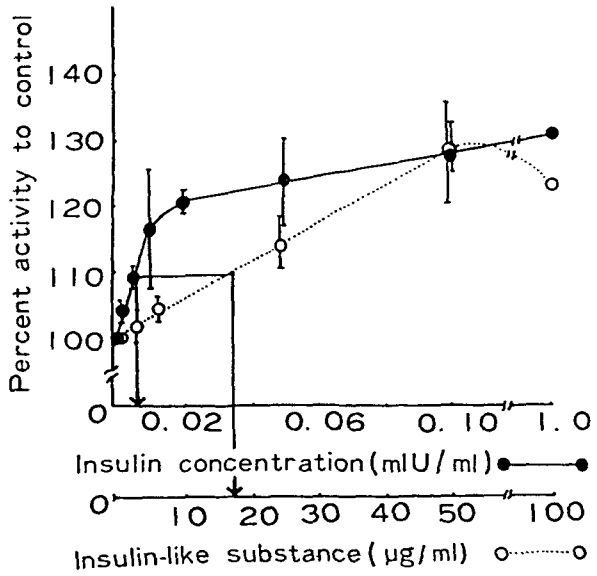

Fig. 7. Effect of insulin-like peptide on lipogenesis from glucose in fat cells

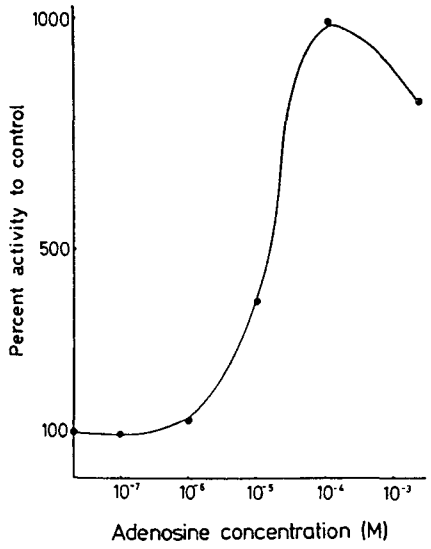

Fig. 8. Effect of adenosine on lipogenesis from glucose in fat cells

demonstrated stimulatory effects on lipogenesis from glucose in fat cells (Fig. 7,8). Namely, the peptide and adenosine possess insulin-like activities such as inhibition of lipolysis and stimulation of lipogenesis. It is well known that insulin secretion and its actions are disturbed in diabetic patients. The fact that pathological conditions of diabetic patients were improved by administrating red ginseng, containing the peptide and adenosine, might be partly explained by red ginseng-induced stimulation of insulin actions in the patients.

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