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Studies on dencichine in Korean red ginseng

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Abstract

Korean red ginseng powder was found to contain dencichine (β-*N*-oxalo-L-α, β-diaminopropionic acid) which was isolated by successive column chromatographies on CM-Sephadex C-25 and Mono Q. We examined the effect of dencichine on the contraction of guinea pig aorta. Although it did not induce contraction or relaxation in the absence of histamine or norepinephrine, it enhanced histamine-induced contraction but not a norepinephrine-induced one. On the other hand, it had no effect on platelet aggregation induced by epinephrine, ADP, thrombin or collagen. It also did not inhibit the angiotensin converting enzyme (ACE).

Key words *Panax ginseng*, dencichine.

Abbreviations ^{1}H-NMR, proton nuclear magnetic resonance; FPLC, Fast Protein Liquid Chromatography; HEPES, *N*-2-hydroxyethylpiperazin-*N*-2-ethanesulfonic acid; ACE, angiotensin converting enzyme; ADP, adenosine diphosphate.

Introduction

Panax ginseng has been used for more than 5,000 years as an herbal medicine in China.[1] In the oldest Chinese Pharmacopea "Shen Nong Ben Tsao Ching", it is claimed to be a divine herb for retaining youth and prolonging life.

Research during the last 20 years has shown that ginseng has the following medical effects: it is an effective defense against stress, and it increases basic metabolism, recovery from fatigue, activation of the central nerves, and stimulation of internal secretions, development of resistance to cancer and diabetes, contributes to the lowering of blood presure, and the secretions of histamine and serotonin, *etc*.[2]

There have been extensive physiological and biochemical studies on the mechanisms of the effects of ginseng in the body. Most of these studies have been on the saponin of ginseng.[3-5] Recently however, we have isolated physiological active substances from non-saponin fractions of Korean red ginseng. One was an acid polysaccharide[6] that inhibited the lipolytic action of toxohormone-L in ascites fluid of sarcoma 180-bearing mice. In addition, we found that Korean red ginseng contained adenosine and pyroglutamic acid, which inhibited epinephrine-induced lipolysis and stimulated insulin-mediated lipogenesis from glucose.[7]

Kosuge *et al.* reported that dencichine was isolated from Sanchi Ginseng Radix as a hemostatic principle.[8] We supposed that dencichine was commonly found in Panax genus including Korean red ginseng.

The present research describes isolation of dencichine from Korean red ginseng powder and examination of its biological activities.

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Materials and Methods

Materials : Red ginseng powder (*Panax ginseng* C.A. MEYER) was kindly provided by Nikkan Korai Ninjin Co. (Kobe, Japan) and Korean Ginseng and the Tobacco Research Institute (Deajeon, Korea). Histamine dihydrochloride was purchased from Wako Pure Chemical Industries (Osaka). Noradrenaline was from Sankyo Co. (Tokyo) and CM-Sephadex C-25 from Pharmacia (Sweden).

Chemical analysis : Paper chromatography was performed on filter paper (ADVANTEC 2) using BuOH/AcOH/H_2O/EtOH (4/1/2/1, v/v) as developing solvents and ninhydrine/AcOH as detecting spray. Dencichine was detected at Rf 0.20. FPLC was carried out with an anion exchange column (MONO Q HR 5/5, Pharmacia). The effluent was detected by optical density at 214 nm which was the shortest wavelength of the detector. Gradient elution was carried cut from 0.01 M to 0.35M NH_4HCO_3. ^1H-NMR spectra were recorded at 270 MHz in a JEOL GSX-270 spectrometer in D_2O. Chemical shifts are expressed in δ ppm relative to internal HDO (δ 4.70).

Animals : Male Hartlay guinea pigs (300−350 g) and male Wistar-King rats (150−200 g) were used.

Measurement of constriction of aorta : The descending thoracic aorta was excised from male guinea pigs and freed of adhering fat and connective tissue. A transverse strip of 15 mm length and 1.5−2.0 mm width was made by cutting the aorta on a ring and was mounted for isometric recording of tension in an oxygenated Tyrode solution. The solution was kept at 37°C and bubbled with air, and the mechanical response of the strip was recorded with an isotonic transducer (Nippon Koden SB-IT). The strip was equilibrated for 2 hr under 1 g resting tension. Then norepinephrine (10^{-7} g/ml) or histamine (10^{-7} g/ml) was applied until the contractile response of the strip attained a constant level. A solution of dencichine was introduced 1 min before application of norepinephrine or histamine.

Platelet aggregation test : Venous blood was collected from healthy volunteers in tubes containing one-tenth volume of 3.8 % sodium citrate. The blood was centrifuged at 150 × *g* for 10 min to obtain platelet-rich plasma (PRP). For examination of dencichine-induced platelet aggregation, 50 μl of dencichine solution dissolved in Ca^{2+}-free Tyrode's buffer, pH 7.4, containing 5 mM HEPES was added to 450 μl of PRP at 37°C with constant stirring at 1,000 rpm in a Bryston-aggregometer and aggregation was measured. For examination of the effects of dencichine on platelet aggregation induced by various agonists, 50 μl of dencichine solution or buffer (control) was added to 400 μl of PRP, and 3 min later 50 μl of an agonist solution in the same buffer was added. In this experiment, the following agonists were used : epinephrine (0.05 μM), ADP (0.2 μM), thrombin (0.01 U/ml) and collagen (0.02 μg / ml). These concentrations of each agonist were just below their thresholds for aggregation. Merely spontaneous elevation of light transmission was observed at the concentrations of these agonists.

Measurement of inhibitory effect on ACE activity: ACE was isolated from rat lung by the method of Takada *et al.*[9] and dissolved in 100 mM phosphate buffer containing 300 mM NaCl (PBS, pH 8.3).

A mixture of 2.5 mM Hip-His-Leu (0.15 ml) and ACE (0.1 ml ; ACE activity : 6.38 units/mg protein, 1 unit : 1 μmole hippuric acid/min/ml of reaction mixture) was incubated with or without test substances at 37°C for 30 min in a final volume of 0.35 ml. The reaction was stopped by adding 1 N HCl (0.25 ml), and the mixture was extracted with ethyl acetate (0.20 ml). The ethyl acetate phase (1.0 ml) was evaporated, and the residue was dissolved in water (2.0 ml). Then free hippuric acid was determined from its ultraviolet (UV) absorption at 228 nm.

Results

A sample of 100 g of red ginseng powder were mixed with 1 *l* of MeOH at room temperature for 24 hr, and then centrifuged at 10,000 × *g* for 30 min. The precipitate was extracted twice with 1

l volumes of distilled water at room temperature for 24 hr periods, centrifuged at 10,000 × *g* for 30 min, and then subjected to suction filtration (ADVANTEC 5 C). The filtrate was concentrated *in vacuo*. The resulting solution was extracted three times with an equal volume of *n*-BuOH saturated with water. The resultant water-soluble portion which was then evaporated to dryness *in vacuo* dissolved in a small amount of distilled water and applied to a column (column, 2.2×40 cm) of CM-Sephadex C-25. Elution was carried out with distilled water. The eluate was subjected to analysis with paper chromatography. The fraction possibly containing dencichine was then subjected to Mono Q (anion exchange) FPLC. The fraction from tube No. 23 to 29 which might contain dencichine was collected and evaporated to dryness *in vacuo* to yield 30 mg of a compound (Fig. 1).

The compound showed maximal absorption at 203 nm and gave a violet color with ninhydrin. The ^{1}H-NMR (D_2O, 270 MHz) spectrum of the compound showed signals of 3 protons, δ 3.83 (1 H, dd, J=3.66, 7.94 Hz), 3.72 (1 H, dd, J=3.66, 14.64 Hz) and 3.56 (1 H, dd, J = 7.94, 14.64 Hz). These spectral data were the same as those of an authentic sample of dencichine.

Dencichine enhanced histamine-induced contraction of guinea pig aorta but not the action of norepinephrine although it did not induce contraction or relaxation in the absence of histamine or norepinephrine (Table I). It did not inhibit ACE activity (Fig. 2). At a concentration of 200 μg/ml it also had no effect on platelet aggregation induced by epinephrine, ADP, thrombine or collagen (Fig. 3). Furthermore, it did not have any effect on arachidonate metabolism in blood platelets (data not shown).

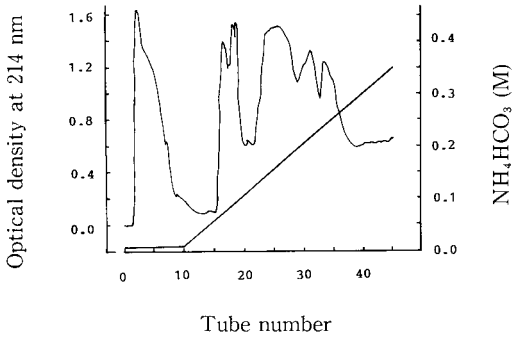

Fig. 1 Fast protein liquid chromatography of dencichine from Korean red ginseng.
Detection : UV, 214 nm ; column : Mono Q ; mobile phase : 0.01 M NH_4HCO_3 – 0.5 M NH_4HCO_3 ; flow rate : 1 ml/min. Fractions of 1 ml of effluent were collected. Dencichine was detected in tube No. 23–29 by paper chromatography (The details are shown in the "Materials and Methods".).

Table I Effects of dencichine on histamine- and norepinephrine-induced constriction of guinea pig aorta.

Agonist	Dencichine (μg/ml)	% of control
Histamine	0	100
(0.54 μM)	1	122
	10	148
	50	174
	100	191
Norepinephrine	0	100
(0.59 μM)	100	102

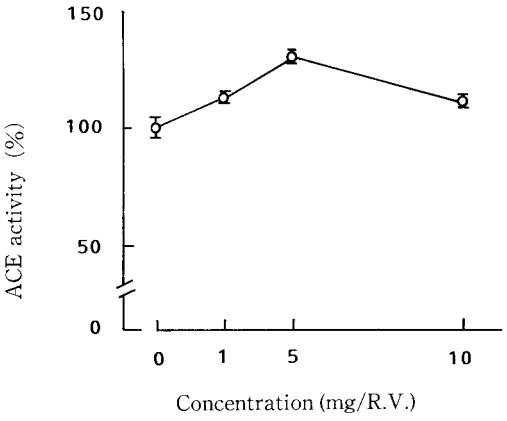

Fig. 2 Effect of dencichine on ACE activity.

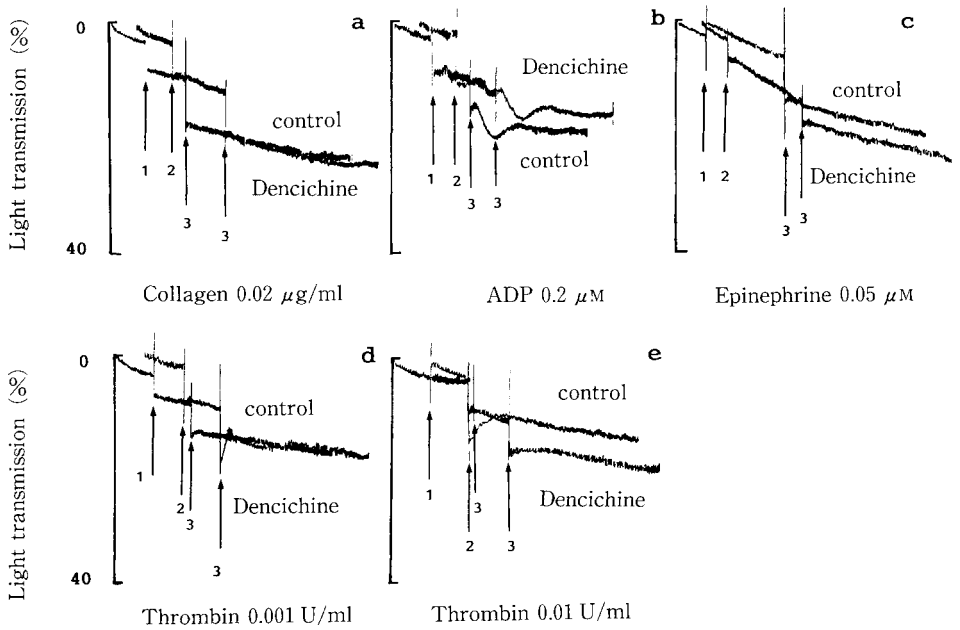

Fig. 3 Effect of dencichine on platelet aggregation.
1) buffer ; 2) dencichine ; 3) collagen 0.02 μg/ml (a), ADP 0.2 μM (b), epinephrine 0.05 μM (C), thrombin 0.001 U/ml (d) or thrombin 0.01 U/ml (e).

Discussion

Dencichine was first isolated from *Lathyrus sativus* by Rao *et al.* as neurotoxin.[10] Then, Kosuge *et al.* found that Sanchi Ginseng Radix contained dencichine and suggested that dencichine was one of the hemostatic components in Sanchi ginseng. These findings afford a possibility that other Panax genus might contain dencichine. In the present communication, we first succeeded in isolating 30 mg of dencichine from 100 g of Korean red ginseng powder with CM-Sephadex C-25 and Mono Q column chromatographies. Then, we tried to examine the biological activities of dencichine. Dencichine failed to inhibit ACE activity. Although it was reported that dencichine was an antihemorrhagic principle in Sanchi Ginseng Radix, this substance did not have any effect on platelet aggregation induced by epinephrine, ADP, thrombin or collagen. Furthermore, we found that it did not exert any effect on arachidonate metabolism in blood platelets. Therfore, it seems likely that other components including degradating products of dencichine in Sanchi Ginseng Radix may cause an antihemorrhagic action.

In the present experiment, we found that dencichine enhanced histamine-induced contraction of guinea pig aorta but not the action of norepinephrine. Both histamine and norepinephrine are known to induce aorta contraction by elevating the free calcium concentration in smooth muscle cells.[11] Therefore, the enhancing action of dencichine on histamine-induced aorta contraction cannot be explained by modulation of the common mechanism of calcium movement, but must be due to change of some specific site of action of histamine in aorta smooth muscle. Experiments are now in progress on the mechanism of the enhancing action of dencichine.

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和文抄録

紅参中にデンシチン（β-*N*-oxalo-L-α, β-diaminopropionic acid）が含まれていることを明らかとし，CM Sephadex C-25 および MONO Q カラムクロマトグラフィーにより順次精製し単離した。デンシチンのモルモット血管収縮に及ぼす影響を検討したところ，ヒスタミン及びノルエピネフリン非存在下では収縮も弛緩も引き起こさなかったが，ヒスタミンの収縮作用を増強しノルエピネフリンの作用には影響を及ぼさなかった。一方，デンシチンはエピネフリン，ADP，トロンビン，コラーゲンによる血小板凝集には何ら影響を及ぼさなかった。また，アンジオテンシン変換酵素 (ACE) を阻害しなかった。

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