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Antihypertensive Activities of Diterpenoid (16,17-dihydroxy-16-β-(−)-kaurane-19-oic acid) in *Siegesbeckiae pubescens* Against Okamoto-Spontaneously Hypertensive Rats*

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희첨 성분중 디터핀 물질이 오까모토 고혈압 모델 쥐에 미치는 혈압 강하작용

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As a folkloric medicine, *Siegesbeckia pubescens* has been used for treatment of brain stroke and hypertension. Diterpene compound, 16,17-dihydroxy-16-β-(−) kaurane-19-oic acid, was isolated from the plant. Its potential antihypertensive activity was evaluated againstan animal model of hypertension, Okamoto-SHR. When diterpene compound with dose of 50 mg/kg/day was orally administered, it exhibited mild antihypertensive activity comparable with propranolol administration dose of 75 mg/kg/day.

Introduction

Folkloric medicinal plants, *Siegesbeckia orientalis* and *Siegesbeckia pubescens* (Compositae) have been widely used in many Asian countries for various medical treatments such as hypertension and inflammation. Phytochemical works on these plants have progressed and were reported elsewhere.[1,3,4]

Two chemical constituents in these plants are melampolides and kaurane type diterperpenoids.[1,4] Recently the structure of ent-kaurane type diterpene isolated from *Siegesbeckia pubescens* was revised and it was reported elsewhere.[3]

Previously authors reported the potential antihypertensivie activity of this diterpene compound (16,17-dihydroxy-16-β-(−)-kaurane-19-oic acid) against an experimental model of hypertension which was produced by ligature of kidney

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in rat.[1] Our present study aims to ascertain such antihypertensive activity of this diterpene compound isolated from *Siegesbeckia pubescens* by using the animal model of Okamoto-spontaneously hypertensive rat developed in Japan.[7] This Okamoto-SHR animal model was extensively studied with respect to its pathogenesis.[6,7,8,9] With this connection, Okamoto-SHR was selected for our present study with diterpene compound isolated from *Siegesbeckia pubescens.*

Meterials and Methods

Isolation of diterpene compound: Phytochemical studies and isolation techniques in detail were reported previously.[1] In brief description, plant was collected in Korea and total 12 kg of dried plant was percolated with methanol at room temperature. After evaporation of methanol, the residue was suspended in water, then this solution thoroughly mixed with ether and ether layer was repeatedly washed with 8% $NaHCO_3$ and with 5% NaOH. Then the solution was again washed with minimum amount of ethylether. The precipitate was formed when the solution was acidified with 10% HCl. This precipitate was recrystallized with ethylacetate/methanol. Total 7 g of crystal yielded. The chemical properties of the crystal formed, 16, 17-dihydroxy-16-β-(−)-kaurane-19-oic acid, was reported previously and it will be called as the diterpene compound B, hereafter. Brief description of procedures was shown in Fig. 1.

Okamoto-SHR: This animal model was developed by professor and Dr. Kozo Okamoto of Kinki University in Japan. Four male and four female rats(B_2, 466779, (780)♂; 466786, (787)♂ and B_2, 466815, (816)♂; 466822 (823)♂, respectively) were kindly donated in 1978 by Professor and Dr. Kozo Okamoto. These rats

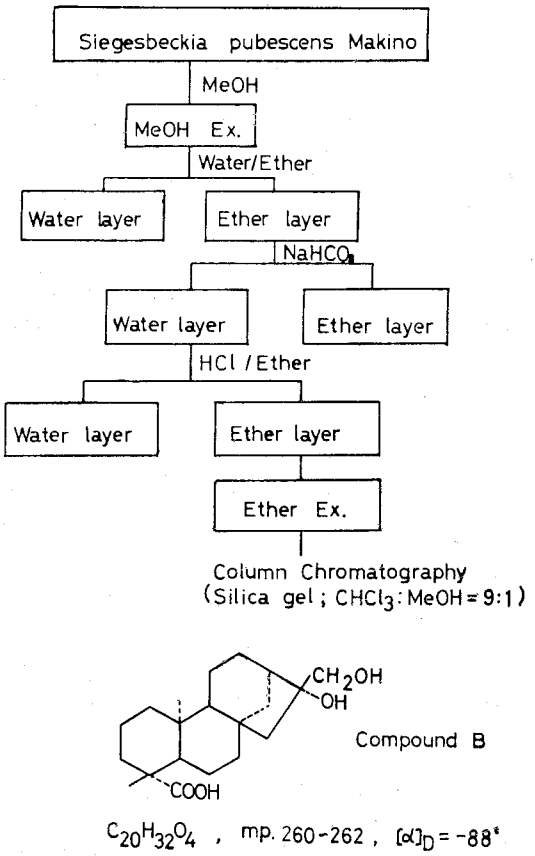

Fig. 1. Isolation of diterpene compound and its structure

have been breeded in our laboratory for the present experiment.

Measurement of blood pressure: Special device was designed to measure systolic blood pressure by using electrosphygmograph having pneumatic pulse attachment. The figure 2 shows our device used in the experiment.[2] All rats were accomodated in air conditioned room for a week before experiment. Then each rat was placed in thermo regulatory box with heater and was preconditioned at 45±5°C for 15 min. before measuring blood pressure at rat tail Okamoto-SHR rats were divided into three group as a positive control received propranolol, known β-blocker, the other two groups received the diterpene compound B with dose

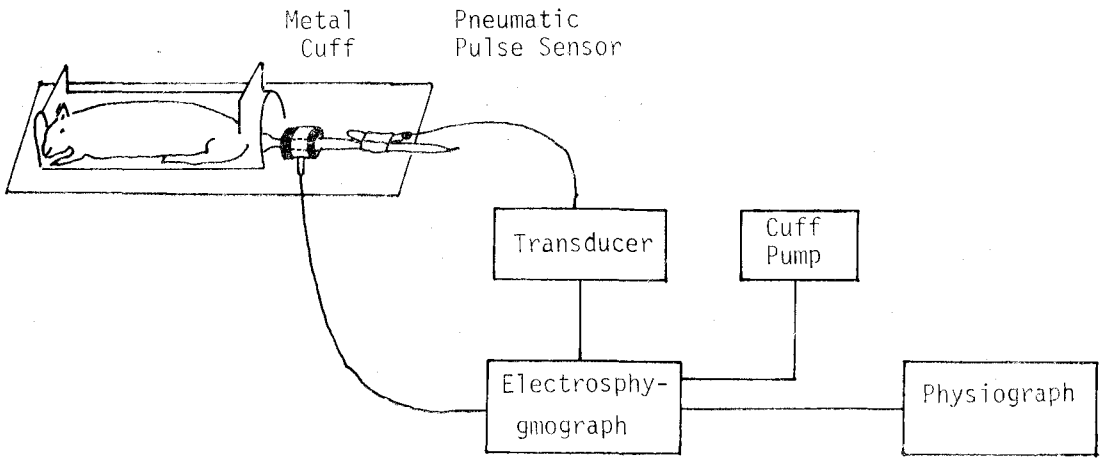

Fig. 2. Schematic diagram of experimental system to record the indirect blood pressure from unanesthetized spontaneously hypertnsive rat.

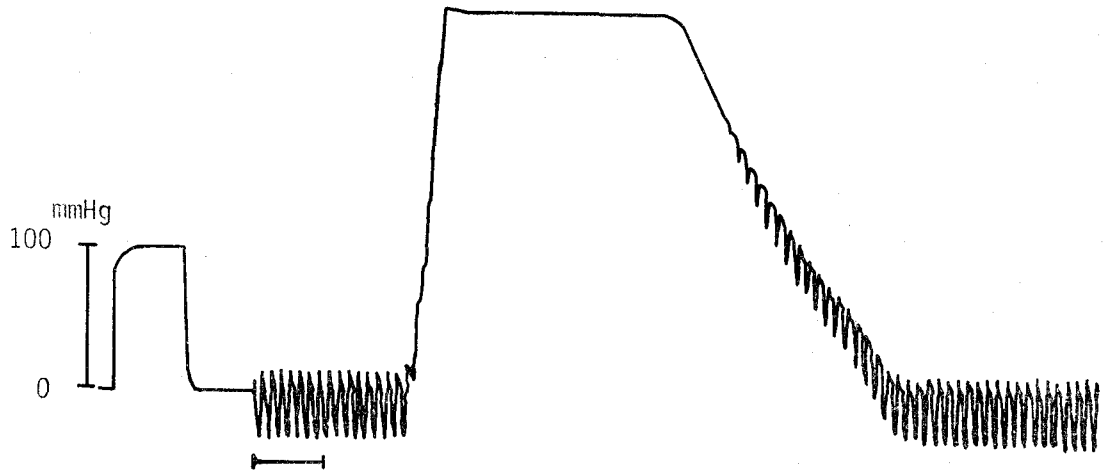

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Fig. 3. Typical recording of indirect systolic blood pressure taken from the tail of a spontaneously hypertensive rat.

ranges from 20 to 50 mg/kg. Typical recording of blood pressure was shown in Fig. 3.

All drugs were suspended in physiological saline with adding few drops of tween 80. For one week before day 0, blood pressure of all rats were measured. Blood pressures in day 0 indicate the average values of the group consisting 6 rats. From day 1 to day 8, all rats received orally drug once daily for 8 days. On day 4, 6 and 8, blood pressure of each rat was measured. Data are shown in Table 1. On day 0, average blood pressure of each group appeared to be 180 mmHg, 196 mmHg, and 200 mmHg, respectively. These blood pressure values seem to be in good agreements with the data previously reported.[6,8] Generally, Okamoto-SHR rats exhibited significantly elevated blood pressure than normal Wistar rat did.

However, the change of blood pressure of individual rat during day also shows time dependent fluctuation with circardian rhythm within range of 30 mmHg.[6] Therefore, caution should

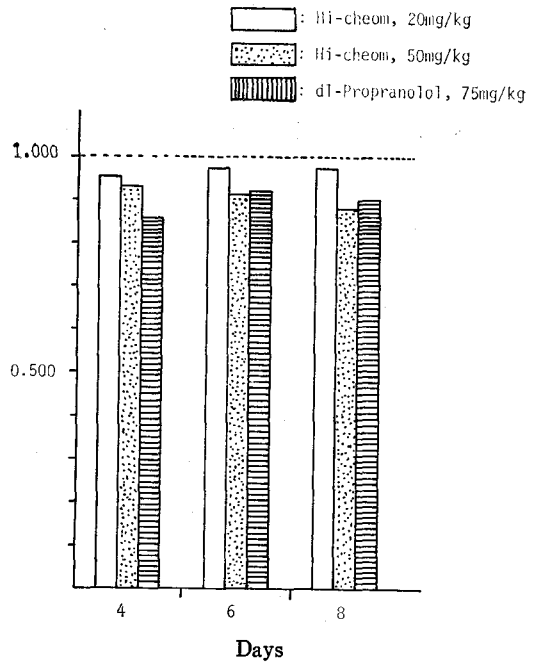

Fig. 4. Fractional changes on blood pressure of SHR. The height of bar was presented as fraction versus the control value.

be paid to evaluate the antihypertensive activity of a certain drug, especially very mild action with long acting one. Because, as the data in Table I shows, the propranolol, dose of 75 mg/kg/day which is several times higher dose than human dose, appeared to exhibit only reduction of 20 mmHg. This value may fall within fluctuation range of Okamoto-SHR blood pressure.[6,8] However, the trend of antihyper tensive activity of propranolol could be evaluated during the experiment with careful measurement. The diterpene compound B isolated from Hi-cheom, Korean name of *Siegesbeckia pubescens* appeared to exhibit blood pressure lowering trend at dose of 50 mg/kg/day, while slight reduction of blood pressure was observed at dose of 20 mg/kg/day. As the figure 4 of fractional representation of activities showed, at dose of 50 mg/kg/day, the diterpene compound B exhibited almost same degree of antihypertensive activity compable with 75 mg/kg/day dose of propranolol. However, it is uncertain whether this diterpene compound B is only responsible for antihypertensiue action of Hi-cheom which has long been used as an ayruvedic drug. Recently, Kim and Ko reported that total ethanol extract of Hi-cheom showed significant antihypertensive activity in rabbit, but its effect was not influenced by propranolol administration.[5] As the data in Table I shows, very high dose of propranolol exhibited only reduction of 20 mmHg, but reserpine appeared to reduce blood pressure of rat profoundly (unpublished). In addition, it was reported that this animal model showed lowered basal renin activity, while, elevated catecholamine concentration.[9] With this connection, propranolol may not be proper for a positive control. There is a possibility of which diterpene compound B itself may not very effective for lowering blood pressure. And it is noteworthy that the Hi-cheom as a folkloric

Table. I. Effects of Hi-cheom compound B and dl-propranolol on systolic blood pressure (mean±S.D.) of spontaneously hypertensive rats. (B.P.unit : mmHg)

Group		Days	0	4	6	8
Hi-cheom	20 mg/kg	B.P.	180±15.9	171±5.0	175±7.0	175±18.5
		Fraction	1.000	0.950	0.972	0.972
Hi-cheom	50 mg/kg	B.P.	196±11.9	183±10.3	180±15.6	173±17.3
		Fraction	1.000	0.934	0.918	0.833
Propranolol	75 mg/kg	B.P.	200±9.8	171±13.4	184±16.5	180±15.4
		Fraction	1.000	0.855	0.920	0.900

medicine has been used for purpose of treating stroke rather than antihypertensive agent. In addition, this animal model appeared to be very sensitive to antihypertensive agents affecting directly catecholamine metabolism.[9]

In present study, we observed mild antihypertensive activity of diterpene compound B, so further study is in progress by employing different dose-schedule, long term administration of this compound and other positive control agent like reserpine, because of its mild action, low solubility in water and the characteristic properties of the animal model.

Acknowledgement

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