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Inhibition of Serotonin Release by Lipophilic Fraction From Korean Red Ginseng

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Abstract□Lipophilic fractions (LF) from *Panax ginseng* C.A. Meyer strongly inhibited human platelet aggregations induced by thrombin. When platelets were prelabeled with 5-Hydroxy[G-^{3}H]-tryptamine (serotonin) and then stimulated by thrombin, LF inhibited the release of serotonin in a dose-dependent manner. From this result, we suggest that LF have antiplatelet and antimigraine functions by inhibiting the release of serotonin.

Key words□Liphophilic fraction, Korean red ginseng, inhibition of human platelet aggregations, inhibition of serotonin release.

Introduction

Serotonins are usually found in the gastrointestinal tract, platelets, and central nervous system in mammalians. The dense bodies in platelet are the main deposits when the concentration of serotonins is calculated by per protein contained in a cell.[1] Being an autacoidal characteristics, serotonin stimulates the platelet aggregations as concomittantly released when platelet is aggregated. Because thrombosis is resulted mainly from the irreversible aggregations which are intimately related with the serotonin release[2-5] and migraine is also caused when serotonin is released,[6,7] the inhibition of the serotonin release by LF is associated with antiplatelet and antimigrainous functions. In this paper, we discussed a new possibility of LF as an antiplatelet drug.

Materials and Methods

1. Materials

5-Hydroxy[G-^{3}H]-tryptamine(creatinine sulphate) was purchased from Amersham Life Science Co., and the other chemical reagents were obtained from Sigma Chemical Co.

2. Preparation of lipophilic fraction from *Panax ginseng* C.A. Meyer and its thin layger chromatogram

500 g of Korean red ginseng were ground into fine powder by a cut mill and has been deposited in 2500 m*l* of petroleum ether for 7 days. Then, it was extracted 3 times with petroleum ether at room temperature and concentrated with vaccum evaporator. The resulting concentrated sample (20 mg) was loaded on to the silicic acid column (dimeter : 1.5 cm, distance 20 cm) and successively eluted with 100 m*l* of hexane/diethylether (95 : 5, v/v), chloroform/methanol (4 : 1, v/v), chloroform/methanol (3 : 2, v/v), chloroform/methanol (1 : 4, v/v), chloroform, chloroform/acetone (9 : 1, v/v), acetone and methanol in order. The 100 m*l* of each eluate and petroleum ether extracts were concentrated and 10 μ*l* of sample were spotted on TLC and developed with petroum ether/diethyl ether (7 : 3, v/v) in order to identify the kinds of lipids. X fraction was scrapped from TLC and was extracted with chroloform/methanol (1 : 2, v/v). The extract was concentrated with evaporator and dissolved in chloroform/methanol (1 : 2, v/v). 10 μ*l* of X fraction

was spotted on TLC plate and developed in chloroform/diethyl ether (1 : 1, v/v). X_1 and X_2 fractions were scrapped, extracted with chloroform/methanol (1 : 2, v/v) and concentrated with evaporator. All fractions were dissolved in the dimethylsulfoxide (DMSO) to be used in the experiment.

3. Preparation of washed platelets

Platelet-rich plasma (PRP) obtained from the antecubital vein of normal human volunteers, was purchased from Taejon Red Cross Blood Center. During PRP preparation, blood was anticoagulated with CPD solution (sodium citrate, NaH_2PO_4, glucose, adenine mixture; Korea Green Cross Pharm.). PRP was centrifuged at 125 x g for 10 min to remove red blood cells, and was washed twice in Tris-citrate-bicarbonate buffer (pH 6.5,[8] containing 2 mM EDTA) by centrifugation at 1,100 x g for 10 min. Because EDTA has an inhibitory action on platelet aggregation, the washed platelets were recentrifuged twice with suspending buffer (pH 6.9,[8] without EDTA). Finally, platelet numbers were adjusted to 5×10^8 cells/m*l* in the suspending buffer. All the above procedures were carried out at 25℃ to avoid platelet aggregation by cold condition.

4. Measurement of platelet aggregation

The washed platelets were preincubated in a cuvette with gentle stirring for 3 min at 37℃. Each reaction cuvette contained 2 mM $CaCl_2$ with or without a testing material. Platelets were stimulated by 0.1 units of thrombin/m*l* for 5 min with gentle stirring. Aggregation was measured with absorbance at 660 nm using a uv/visible spectrophotometer (Beckman DU-6). Transmisson (T) was calculated from following formula.

$$T=\frac{1}{10^{\Delta A}}$$

$$\Delta A=5A-3A$$

5A = absorbance when aggregation reaction has occurred for 5 min

3A = absorbance when preincubation was carried out for 3 min

Suspending buffer was used as reference (absorbance 0). All fractions were dissolved in DMSO, therefore, its pure activity was calculated by substracting that of DMSO.

5. Measurement of serotonin release

One μM of 5-hydroxy[G-^{3}H]tryptamine (creatinine sulphate) was added to human platelet rich plasma (PRP). 5-Hydroxy[G-^{3}H]tryptamine has a fluorescent property, so that PRP was wrapped with aluminum foil and incubated at 37℃ for 60 min. 5-hydroxytryptamine(serotonin)-loaded platelets were prepared as described in "perpartion of washed platelets". The release of serotonin was measured by the method of Costa and Murphy.[9]

Results

1. TLC pattern of lipophilic fraction (LF)

The petroleum ether extract from Korean red

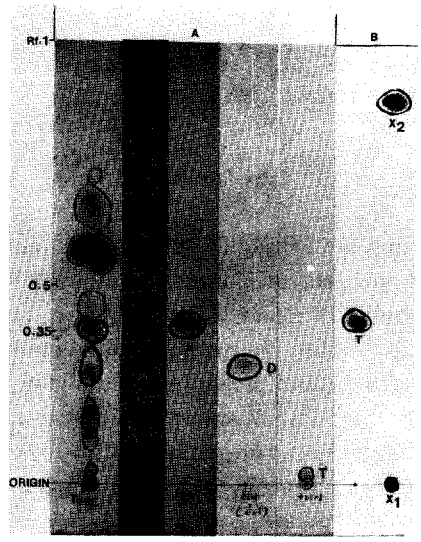

Fig. 1. Thin layer chromatogram of petroleum ether extract from red ginseng and its subfractions. All samples were spotted on the glass plate coated with silica gel (particle size; 5~17 μm, layer; 250 μm) and then the plate was developed in the solvent systems as follows; A side : petroleum ether/diethyl ether (7 : 3, v/v), B side : chloroform/diethyl ether (1 : 1, v/v). To identify the spots of separated compounds, the plate was air dried and then exposed to I_2 vapor. 1 : petroleum ether extracts. 2 : fraction eluted by hexane/diethylether (95 : 5, v/v). 3 : fraction eluted by the mixture of chloroform and methanol (ref. : method). 4(N) : panaxynol standard. 5(D) : panaxydol standard. 6(T) : panaxytriol standard.

ginseng root was developed on TLC plate with petroleum ether/diethyl ether (7 : 3, v/v) solution. The spots of polyacetylene compounds such as panaxynol, panaxydol, and panaxytriol were identified on the TLC plate as shown in Fig. 1 (lanes 1~6). When the extract with petroleum ether (Fig. 1, lane 1) was eluted with various solvent combination as described in the methods, X fraction eluted with chloroform/methanol (4 : 1, 3 : 2 and 1 : 4, v/v), chloroform, chloroform/acetone (9 : 1, v/v), acetone and methanol was only identified on the origin of TLC plate in the all eluates (Fig. 1, lane 3). Polyacetylene compounds of LF were eluted in hexane/diethylether (95 : 5, v/v) (Fig. 1, lane 2). However, because the contamination of panaxytriol on the origin, we developed the X fraction in the solvent system of chloroform/diethyl ether (1 : 1. v/v) again. As shown in Fig. 1B, panaxytriol was not detected, but a polar lipophilic spot (X_1) and non polar lipophilic one (X_2) were detected on origin and R_f 0.8 on the plate, respectively. At present, the characteristics and structure of separated compounds have not been determined and further study is required.

2. Effects on platelet aggregations and the release of serotonin

Table 1. Inhibition of human platelet aggregation induced by thrombin

	Degrees of aggregation (%)	Inhibition of aggregation
Thrombin (1 μ/m*l*)	82 (n−2)	0
Thrombin (1 μ/m*l*)+ LF (100 μg/m*l*)	10.5± 1.1 (n−4)	87.2± 1.34 (n=4)

Aggregation reactions were performed as described in the method. X fraction observed in Fig. 1 was designated as LF in this table. The data are given as mean± S. D. (n−2~4).

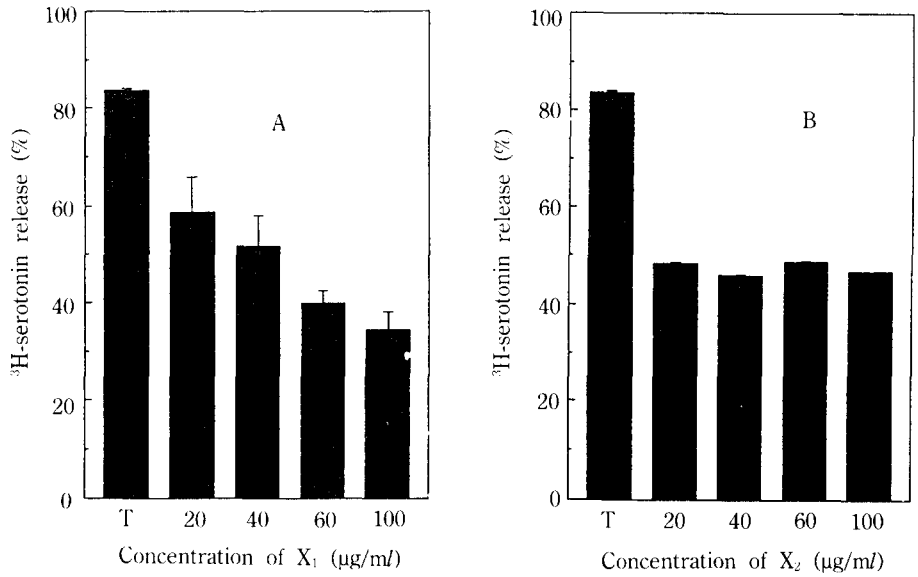

Fig. 2. Inhibition of lipophilic frction from Korean red ginseng on serotonin release in the thrombin induced platelet aggregation. A : Inhition of X_1 fraction. B : Inhibition of X_2 fraction. Human platelets (10^8/m*l*) loaded with [^{3}H] serotonin were preincubated at 37℃ for 3 min with addition of 2 mM $CaCl_2$ and X_1 or X_2, and then stimulated by the 2 units of thrombin/m*l* for 5 min to release the serotonin. The reaction was terminated by the addition of 16.5% formaldehyde and the reaction tubes were centrifuged at 1,100 x g for 10 min immediately. The 100 μ*l* of supernatnt were transferred into the vials containing 10 m*l* of Scint A-XF (packard) to measure the cpm by a liquid scintillation counter. The total cpm value from platelets (10^8/m*l*) was 140,000~150,000. The degree of serotonin release by each fraction is expressed as percentage. All the data are compensated by DMSO where NSF is dissolved. The data are given as the mean± S.D. (n=4). T : Thrombin (2 u/m*l*).

When human platelets were stimulated by thrombin (1 u/ml), aggregations were occurred by 82%. However, when plateletes were preincubated for 2 min with an addition of X-fraction (100 μg/ml), the aggregations were inhibited up to 87.2% (Table 1). As shown in Fig. 2, when plateletes were prelabled with ^{3}H-serotonin and stimulated with 2 u/ml of thrombin, 83% of serotonin were released out of human plateletes. But X_1 and X_2 fractions inhibited the release of serotonin in a dose dependent manner (Fig. 2A, B). X_1 fraction was stronger than X_2 fraction in the inhibition of serotonin release.

Discussion

When platelets are stimulated by thrombin, thromboxane A2(TXA2) is produced and platelets are aggregated by TXA_2.[10] Especially high concentrations of thrombin (1~5 u/ml) stimulate the release of serotonin, and cause the irreversible aggregation.[2-5] The aggregations induced by high dose of thrombin were not successfully inhibited by inhibiting the production of TXA_2 only.[2] This means that the serotonin was released from platelets by high concentration of thrombin. Indomethacin, a well known antiplatelet drug, is known to not inhibit the serotonin release when the platelets are stimulated with high concentrations of thrombin.[11] But X fraction and its subfractions, X_1 and X_2, inhibited the platelet aggregations and the serotonin release when platelets were induced by high concentrations of thrombin, respectively. Therefore, it can be suggested that X_1 and X_2 fractions from Korea red ginseng inhibit the irreversible aggregation induced by high concentrations of thrombin and possibly inhibit the thrombosis by inhibiting the release of serotonin. Polyacetylene compounds such as panaxynol, panaxydol, panaxytriol were contained in petroleum ether extracts of red gineng. These polyacetylene compounds inhibit the platelet aggregation.[12] In our experiment, X_1 and X_2 fractions of petroleum ether extracts was not polyacetylene compounds. Accordingly, X_1 and X_2 fractions seem to be a novel substances tht inhibit platelet aggregation.

요 약

Panax ginseng C.A. Meyer로부터 제조한 Lipophilic Fractions(LF)은 thrombin으로 유인된 사람 혈소판 응집반응을 강하게 억제시켰다. 5-Hydroxy[G-^{3}H] tryptamine(Serotonin)을 prelabel시켜 thrombin으로 자극시켰을 때, LF는 농도의존적으로 serotonin의 방출을 억제시켰다. 이 결과는 LF가 혈소판으로부터 serotonin의 방출을 억제시키므로써 antiplatelet 또는 antimigraine 작용을 할 수 있다는 가능성을 시사하는 것이다.

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