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Effects of Kakkon-to, Mao-to, Tokaku-joki-to and San'o-shashin-to on prostaglandin E_2 release from C6 rat glioma cells

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Abstract

Glial cells, prostaglandin generating cells in the brain, have a role to maintain neuronal function. We investigated the effects of Kakkon-to (TJ-1, Ge-Ge-Tang, 葛根湯), Mao-to (TJ-27, Ma-Huang-Tang, 麻黄湯), Tokaku-joki-to (TJ-61, Tao-He-Cheng-Qi-Tang, 桃核承気湯) and San'o-shashin-to (TJ-113, San-Huang-Xie-Xin-Tang, 三黄瀉心湯), Kampo medicines (Chinese herbal medicines), on prostaglandin E_2 (PGE_2) release from glial cell line, C6 rat glioma cells, in order to clarify the possible mechanisms of action of these Kampo medicines. A23187, a Ca^{2+} ionophore, released PGE_2 from C6 cells in a time- and concentration-dependent manner. The treatment of the cells with the above Kampo medicines for 10 min resulted in a significant reduction of A23187-induced PGE_2 release. Among the crude drugs composing these Kampo medicines, Puerariae Radix, Glycyrrhizae Radix, Cinnamomi Cortex, Paeoniae Radix, Zingiberis Rhizoma, Scutellariae Radix and Rhei Rhizoma significantly reduced A23187-induced PGE_2 release. Especially, Glycyrrhizae Radix, Zingiberis Rhizoma, Scutellariae Radix and Rhei Rhizoma showed potent inhibition of the PGE_2 release. These results suggest that the four Kampo medicines have inhibitory action on arachidonic acid metabolism in glial cells, and each Kampo medicine contains at least two crude drugs inhibiting PGE_2 release.

Key words prostaglandin E_2, glial cells, Kakkon-to, Mao-to, San'o-shashin-to, Tokaku-joki-to.

Introduction

Prostaglandin E_2 (PGE_2) has pyrogenic activity through the elevation of set point of the hypothalamus which regulates body temperature. Aspirin-like drugs are well known to cause an inhibition of cyclooxygenase resulting in reduction of PGE_2 release.[1] In the central nervous system, PGE_2 has an ability to cause awakening against sleep[2] in addition to fever generation. Among cells composing brain, glial cells including astrocytes and oligodendrocytes have been shown as a major source for producing PGE_2.[3-5] Cytokines, ATP and bradykinin have been shown to cause PGE_2 release from astrocytes.[6-8]

Several Kampo medicines (Chinese herbal medicines) are widely used as analgesic-antipyretics for treatment of the common cold. Among them, Kakkon-to (Ge-Ge-Tang, 葛根湯)[9-11] is the most popular, which is used at an initial phase of the common cold. Kakkon-to consists of 7 crude drugs of Puerariae Radix, Ephedrae Herba, Zizyphi Fructus, Cinnamomi Cortex, Paeoniae Radix, Glycyrrhizae Radix, and Zingiberis Rhizoma. On the other hand, Mao-to (Ma-Huang-Tang, 麻黄湯),[9] another type of Kampo medicine, is also used in the initial phase of the common cold with severe chill and joint pain. Mao-to consists of 4 crude drugs of Ephedrae Herba, Glycyrrhizae

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Radix, Cinnamomi Cortex and Armeniacae Semen. In addition, Tokaku-joki-to (Tao-He-Cheng-Qi-Tang, 桃核承気湯) and San'o-shashin-to (San-Huang-Xie-Xin-Tang, 三黄瀉心湯)[12,13] are used for the treatment of psychotic disorders, such as headache, anxiety, sleeplessness and vertigo. Tokaku-joki-to consists of 5 crude drugs of Glycyrrhizae Radix, Cinnamomi Cortex, Persicae Semen, Rhei Rhizoma and Mirabilite. San'o-shashin-to consists of 3 crude drugs of Scutellariae Radix, Coptidis Rhizoma and Rhei Rhizoma.

For pharmacological evaluation of these drugs, we examined the effects of the above Kampo medicines and their composing crude drugs on PGE_2 release in C6 rat glioma cells. The results obtained suggest that these Kampo medicines have an ability to reduce PGE_2 release and each Kampo medicine contains at least two crude drugs inhibiting PGE_2 release.

Materials and Methods

Materials : Dulbecco's modified Eagle's medium (DMEM) and Eagle's minimum essential medium (EMEM) were purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). Fetal calf serum (FCS) was purchased from General Scientific Laboratory (Los Angeles, CA, USA). A23187 was from Wako Pure Chemical Co. (Osaka, Japan). [^{3}H] PGE_2 (200 Ci/mmol) was from DuPont/NEN (Boston, MA, USA). PGE_2 and an antibody to PGE_2 were kindly donated by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). Kakkon-to (TJ-1, Ge-Ge-Tang), Mao-to (TJ-27, Ma-Huang-Tang), Tokaku-joki-to (TJ-27, Tao-He-Cheng-Qi-Tang) and San'o-shashin-to (TJ-113, San-Huang-Xie-Xin-Tang) were obtained from Tsumura & Co. (Tokyo, Japan). Each Kampo medicine was prepared as follows : A mixture of crude drugs as shown in Table I was extracted with water (285 ml) at 100°C for 1 h. The extract solution was filtered and spray-dried to obtain the dry extract powder. The recovery during the procedure was also shown in Table I. The crude drugs used were Puerariae Radix (root of *Pueraria lobata* OHWI), Zyziphi Fructus (fruit of *Zyziphus jujuba* MILLER var. *inermis* REHDER), Ephedrae Herba (herb of *Ephedra sinica* STAPF), Glycyrrhizae Radix (root of *Glycyrrhiza uralensis* FISCH et. DC.), Cinnamomi Cortex (bark of *Cinnamomum cassia* BUNGE), Paeoniae Radix (root of *Paeonia lactiflora* PALL.), Zingiberis Rhizoma (rhizome of *Zingiber officinale* ROSCOE), Scutellariae Radix (root of *Scutellaria baicalensis* GEORGI), Coptidis Rhizoma (rhizome of *Coptis japonica* MAKINO), Armeniacae Semen (seed of *Prunus armeniaca* L.), Persicae Semen (seed of *Prumus persica* BATSH), Rhei Rhizoma (rhizome of *Rheum Palmatum* L.) and Mirabilite (Na_2SO_4). Each crude drug (10 g) was

Table I The crude drug components of four kinds of Kampo medicines.

	Kakkon-to (TJ-1) (Ge-Gen-Tang) [33 %]	Mao-to (TJ-27) (Ma-Huang-Tang) [14 %]	Tokaku-joki-to (TJ-61) (Tao-He-Cheng-Qi-Tang) [30 %]	San'o-shashin-to (TJ-113) (San-Huang-Xie-Xin-Tang) [26 %]
Puerariae Radix [35 %]	4.0 g			
Zizyphi Fructus [50 %]	3.0 g			
Ephedrae Herba [23 %]	3.0 g	5.0 g		
Glyccyrhizae Radix [27 %]	2.0 g	1.5 g	1.5 g	
Cinnamomi Cortex [8 %]	2.0 g	4.0 g	4.0 g	
Paeoniae Radix [25 %]	2.0 g			
Zingiberis Rhizoma [25 %]	2.0 g			
Scutellariae Radix [32 %]				3.0 g
Coptidis Rhizoma [20 %]				3.0 g
Armeniacae Semen [33 %]		5.0 g		
Persicae Semen [20 %]			5.0 g	
Rhei Rhizoma [27 %]			3.0 g	3.0 g
Mirabilite [−]			0.9 g	

Recovery by extraction was shown as % in [ ] for crude drugs or Kampo medicines.

extracted with water (100 ml) at 100°C for 1 h except Mirabilite. The extract solution was filtered and spray-dried to obtain the dry extract powder. The recovery during the procedure was also shown in Table I. The dried extracts of Kampo medicines or crude drugs were dissolved in 0.9 % NaCl to make a concentration of 10 mg/ml by sonication. The solution was diluted 10 times by the culture medium. Other chemicals or drugs were reagent grade or the highest quality available.

Cell culture : C6 cells were grown in 10 % FCS-DMEM containing 50 U/ml of penicillin and 50μg/ml of streptomycin at the condition of 37°C in air containing 5 % CO_2. The medium was changed every 3 or 4 days.

Assay of prostaglandin E_2 : C6 cells were seeded into 12-well plates at a density of 10^5 cells/well and were used 3-4 days after the subculture. The cells were washed twice with EMEM buffered with 20 mM HEPES, pH 7.35 (EMEM-HEPES), and they were preincubated with Kampo medicines, crude drug or vehicle for 10 min. Then, the cells were incubated with A23187 or vehicle for an additional 10 min in EMEM-HEPES. The reaction medium was collected into an ice-cold tube at the end of incubation. Each medium was stored at -20°C until extraction of PGE_2. PGE_2 was extracted twice with ethyl acetate after acidifying the medium to pH 4.0 by 1 N HCl. Ethyl acetate was evaporated by a stream of N_2 gas at 40°C. PGE_2, which was dissolved in 10 mM Tris-HCl (pH 7.6), was determined by a radioimmunoassay as described previously.[14,15]

Statistics and data analysis : The statistical significance of the difference between values obtained was determined by using Student's t-test.

Results

A23187, a Ca^{2+} ionophore, is known to stimulate prostaglandin synthesis mediated through an activation of phospholipase A_2 following by arachidonic acid liberation in glial cells.[16] In C6 rat glioma cells, A23187 (10 μM) stimulated PGE_2 release in a time-dependent manner (Fig. 1A). A23187-induced PGE_2 release was concentration-dependent with an EC_{50} value of approximately 0.8 μM (Fig. 1B).

The treatment of the cells with indomethacin, a cyclooxygenase inhibitor,[1] for 10 min resulted in potent attenuation of A23187-induced PGE_2 release from C6 rat glioma cells (Fig. 2A), indicating that the release was catalyzed by cyclooxygenase. The treatment of the cells with Kakkon-to, Mao-to, Tokaku-joki-to or San'o-shashin-to for 10 min also resulted in the reduction of A23187-induced PGE_2 release (Fig. 2B), showing that these four Kampo medicines contain the substance(s) inhibiting PGE_2 release.

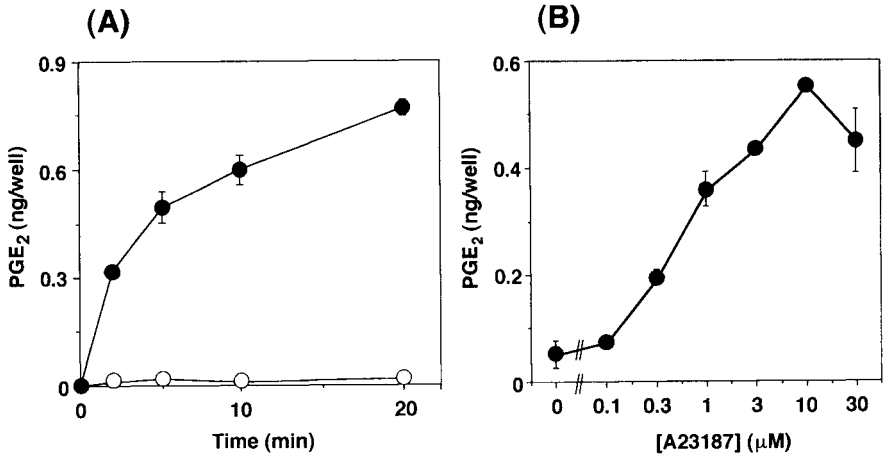

Fig. 1 Effects of A23187 on PGE_2 release from C6 rat glioma cells.
(A) Time course of PGE_2 relese by A23187 (10 μM). ● : A23187, ○ : vehicle. (B) The concentration-dependency of PGE_2 release by A23187. The cells were incubated for 10 min. The PGE_2 in the incubation medium was analyzed by radioimmunoassay. Each point represents the mean ± s.e. of three determinations.

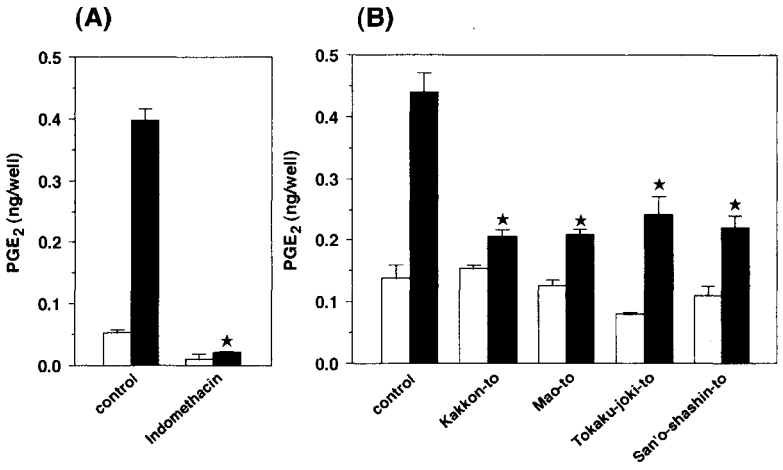

Fig. 2 Effects of indomethacin and four Kampo medicines on PGE_2 release from C6 rat glioma cells.
(A) The cells were incubated with indomethacin (5 μM) for 10 min, then incubated with A23187 (10 μM) for an additional 10 min. (B) The cells were incubated with Kakkon-to, Mao-to, Tokaku-joki-to and San'o-shashin-to (100 μg/ml) for 10 min, then incubated with A23187 (10 μM) for an additional 10 min. The PGE_2 in the incubation medium was analyzed by radioimmunoassay. Open column, without A23187; hatched column, with A23187. Each column represents the mean±s.e. of three determinations. Data were representative in at least two separate experiments. ★: Statistically significant difference from the value with A23187 alone ($p<0.05$).

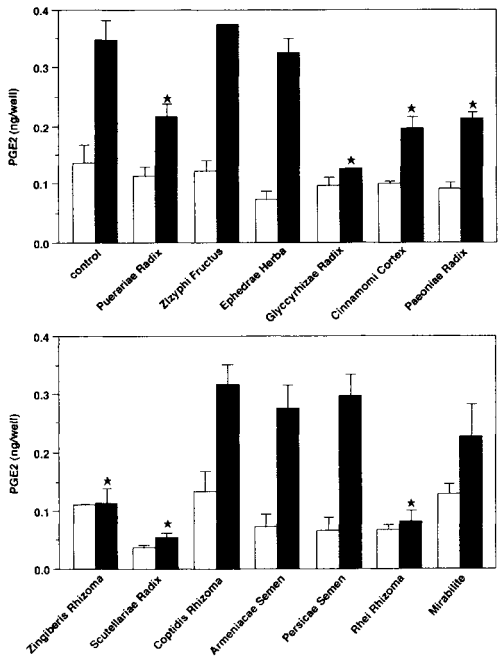

Fig. 3 Effects of crude drugs composed of four Kampo medicines on PGE_2 release from C6 rat glioma cells.
The cells were incubated with each crude drug (100 μg/ml) for 10 min, then incubated with A23187 (10 μM) for an additional 10 min. The PGE_2 in the incubation medium was analyzed by radioimmunoassay. Open column, without A23187; hatched column, with A23187. Each column represents the mean±s.e. of three determinations. Data were representative in at least two separate experiments. ★: Statistically significant difference from the value with A23187 alone ($p<0.05$).

The crude drugs composing these four Kampo medicines were then examined as to whether or not they affect PGE_2 release from C6 rat glioma cells (Fig. 3). Glycyrrhizae Radix, Zingiberis Rhizoma, Scutellariae Radix and Rhei Rhizoma potently inhibited A23187-induced PGE_2 release. Puerariae Radix, Cinnamomi Cortex and Paeoniae Radix inhibited A23187-induced PGE_2 release moderately (Fig. 3). In contrast, Zizyphi Fructus, Ephedrae Herba, Coptidis Rhizoma, Armeniacae Semen, Persicae Semen and Mirabilite had no effect on A23187-induced PGE_2 release (Fig. 3).

Discussion

The present study demonstrates that Kakkon-to, Mao-to, Tokaku-joki-to and San'o-shashin-to clearly inhibit PGE_2 release from glial cell line C6 rat glioma cells. Several crude drugs composing these Kampo medicines inhibit PGE_2 release.

Both Kakkon-to and Mao-to, which are used for the treatment of the common cold, contain Ephedrae Herba, Glycyrrhizae Radix and Cinnamomi Cortex with a different ratio. Since Glycyrrhzae Radix and Cinnamomi Cortex are effective in reduction of PGE_2

release, both Kampo medicines are thought to be useful as antipyretics. In addition to Glycyrrhizae Radix and Cinnamomi Cortex, Puerariae Radix, Paeoniae Radix and Zingiberis Rhizoma in Kakkon-to are effective crude drugs to inhibit PGE_2. Therefore, it is assumed that Kakkon-to has multiple crude drugs to reduce PGE_2 release for recovering from the common cold. Puerariae Radix contains puerarin, which has been shown to possess several pharmacological properties, such as anti-alcoholism[16] presumably by acting on benzodiazepine receptor,[17] β-adrenergic antagonism[18] for effectiveness to anti-myocardial injury,[19] inhibition of xanthine oxidase,[20] and anti-proliferative action.[21] Puerariae Radix is, therefore, a novel crude drug in Kakkon-to to characterize the pharmacological actions specific to Kakkon-to.

Tokaku-joki-to and San'o-shashin-to are used for the treatment of psychotic disorders, such as headache, anxiety, sleeplessness and vertigo. One of physiological effects of PGE_2 is awakening in central nervous system.[2] Furthermore, PGE_2 exerts as an inhibitor of neurotransmitter release.[22-24] The present study demonstrated that Tokaku-joki-to and San'o-shashin-to decreased PGE_2 release from C6 rat glioma cells. These inhibitory effects of both medicines on PGE_2 release may contribute to their pharmacological activities.

Among crude drugs composed of Tokaku-joki-to, Glycyrrhizae Radix, Cinnamomi Cortex and Rhei Rhizoma are effective to decrease PGE_2 release. Persicae Semen had no effect on PGE_2 release, although benzaldehyde that is produced by hydrolysis of amygdalin, a main constituent of Persicae Semen, has a similar chemical structure to aspirin. This was supported by the observation that Armeniae Semen containing amygdalin also had no effect on PGE_2 release (Fig. 2).

San'o-shashin-to consists of three crude drugs, Scutellariae Radix, Coptidis Rhizoma and Rhei Rhizoma. Among them, Scutellariae Radix and Rhei Rhizoma were effective in reduction of PGE_2 release. Scutellariae Radix had the most potent inhibitory effect on PGE_2 release among crude drugs in the present study. Recently our detailed analysis of constituents of Scutellariae Radix indicates that flavonoids, such as baicalein and wogonin, are the main substances to inhibit PGE_2 release through mitogen-activated protein kinase and phospholipase A_2.[26] It has been reported that baicalein, an ingredient of Scutellariae Radix, has several pharmacological actions, such as anti-inflammatory effect,[26] anti-peptide leukotrienes,[27] inhibition of 12-lipoxygenase in platelets,[28] inhibition of platelet aggregation,[29] potentiation of vasoconstriction,[30] anti-tumor effects,[31] inhibition of xanthine oxidase[32] and anti-proliferative effect.[33] The inhibitory effect of PGE_2 release should be added to one of pharmacological actions of Scutellariae Radix.

The present study demonstrated that several crude drugs consisting of the four Kampo medicines showed inhibitory effects on PGE_2 release from C6 rat glioma cells. Each Kampo medicine contains at least two crude drugs that inhibit PGE_2 release. These results may contribute to the interpretation of the pharmacological actions of the Kampo medicines. Further studies will be necessary to clarify the mechanism of these drugs in inhibiting PGE_2 release, and what ingredients are effective.

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和文抄録

中枢において発熱や覚醒に関与すると考えられているプロスタグランジン E_2 (PGE_2) の生成に及ぼす，葛根湯 (TJ-1)，麻黄湯 (TJ-27)，桃核承気湯 (TJ-61) および三黄瀉心湯 (TJ-113) の作用について検討した。C6 ラットグリオーマ細胞では，Ca^{2+} イオノフォアの A23187 によって時間および用量依存的な PGE_2 遊離の亢進が見られた。この亢進は葛根湯 (TJ-1)，麻黄湯 (TJ-27)，桃核承気湯 (TJ-61) および三黄瀉心湯 (TJ-113) の前処置により有意に抑制された。各漢方薬を構成する生薬の作用を検討したところ，葛根湯については，葛根，甘草，桂皮，芍薬および生姜が PGE_2 遊離を抑制した。麻黄湯については甘草および桂皮が抑制を示した。一方，

桃核承気湯では甘草，桂皮および大黄が PGE_2 遊離抑制を示し，三黄瀉心湯では黄芩および大黄が抑制を示した。これらの結果より，葛根湯（TJ-1），麻黄湯（TJ-27），桃核承気湯（TJ-61）および三黄瀉心湯（TJ-113）が中枢神経系のアラキドン酸代謝に影響を与える可能性を示すとともに，それぞれの漢方薬が PGE_2 遊離抑制を起こす少なくとも2種以上の生薬より構成されていることが示された。

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