Antagonism of Saikosaponin-Induced Prostaglandin E₂ Release by Baicalein in C6 Rat Glial Cells

Reiki Kyo,* Norimichi Nakahata,*, a Yasuo Kodama, a Yoichiro Nakai, b Masayoshi Kubo, b and Yasushi Ohzumi a

Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, a
Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan and Kampo and Pharmacognosy Laboratory, Tsumura and Co., b 3586
Yoshiwara, Aminachi, Inashiki, Ibaraki 300–1192, Japan. Received June 7, 1999; accepted September 1, 1999

There are several Kampo medicines (Chinese herbal medicines) containing both Bupleuri Radix and Scutellariae Radix, which are used for the treatment of inflammation. Saikosaponins are derived from Bupleuri Radix, and baicalein is from Scutellariae Radix. The present study was undertaken to investigate the pharmacological interaction of saikosaponin b1 and baicalein in prostaglandin E₂ (PGE₂) release from C6 rat glialoma cells in vitro. Saikosaponin a, b1 and d potently stimulated PGE₂ release, while saikosaponin b2 and c moderately stimulated PGE₂ release. Saikosaponin b1 caused an irreversible elevation of intracellular Ca²⁺ concentration, which was eliminated by removing extracellular Ca²⁺. On the other hand, baicalein inhibited saikosaponin b1-induced PGE₂ release in a concentration-dependent manner. These results suggest that saikosaponins are activators of PGE₂ release, and baicalein is one of the functional inhibitors of PGE₂ release by saikosaponins.

Key words saikosaponin; baicalein; prostaglandin E₂; calcium ion; glial cell

The extracts of Bupleuri Radix are commonly used as a crude drug composed of several Kampo medicines (Chinese herbal medicines), such as Otsuji-to (Yi-Zi-Tang), Sho-saiko-to (Xiao-Chai-Hu-Tang), Dai-saiko-to (Da-Chai-Hu-Tang), Saiko-kei-shi-to (Chai-Hu-Gui-Zhi-Tang), Saiboku-to (Chai-Pu-Tang) and Sairei-to (Chai-Ling-Tang). These Kampo medicines also contain the extract of Scutellariae Radix together with Bupleuri Radix.

Bupleuri Radix contains saikosaponins, which have been shown to exert various biological effects, such as central depression, anti-inflammation, hemolytic action and an antiviral action. Furthermore, Abe et al. reported the inhibitory effect of saikosaponins on hepatic injury induced by D-galactosamine. On the other hand, Scutellariae Radix contains flavonoids such as baicalin, baicalein and wogonin. These flavonoids have unique pharmacological activities, such as anti-inflammatory activity, the inhibition of leukotriene production, inhibition of prostaglandin (PG) synthesis, inhibition of platelet lipooxygenase, inhibition of sialidase, anti-tumor effects on human cancer cell line, anti-proliferative action, the inhibition of xanthine oxidase, and potentiation of smooth muscle contraction.

Gliai cells, which outnumber neurons by about ten to one in the brain, provide both mechanical and metabolic support for neurons. Gial cells are assumed to be an important source of PGs in the central nervous system.

In the present study, we have investigated the effects of saikosaponins on prostaglandin E₂ (PGE₂) release from C6 rat glialoma cells and the pharmacological interactions between saikosaponin b1 and baicalein.

MATERIALS AND METHODS

Chemicals 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 was obtained from General Scientific Laboratory (Los Angeles, CA, U.S.A.). [³²H]PGE₂ (200 Ci/mmol) was from DuPont/NEM (Boston, MA, U.S.A.). PGE₂ and an antibody to PGE₂ were kindly donated by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Fura 2-AM was obtained from Dojindo (Kumamoto, Japan). Triton X-100 was from Wako Pure Chemicals (Tokyo, Japan). EGTA was from Nacalai Tesque (Kyoto, Japan). Collagenase was purchased from Worthington Biochemical Co. (Freehold, NJ, U.S.A.). For purification of saikosaponins and baicalein, the methanol extracts of Bupleuri Radix (root of Bupleurum falcatum L.) or Scutellariae Radix (root of Scutellaria baicalensis Georgi) were partitioned with water and diethyl ether, and then n-butanol. Saikosaponins and baicalein were obtained through the process of silica gel chromatography from the respective n-butanol fraction and the diethyl ether fraction. Saikosaponins and baicalein were dissolved in dimethyl sulfoxide to make a concentration of 50 mM, and used after dilution with medium.

Cell Culture C6 rat glioma cells were grown in F-10 (Ham) medium containing 15% horse serum and 2.5% fetal calf serum in a 37°C humidified incubator in an atmosphere of 5% CO₂ in air, as previously described.

Determination of PGE₂ C6 cells were seeded into 12-well plates at a density of 1.0×10⁴ cells per well. The experiment was performed 2 d after cell seeding. The cells were washed twice with EMEM buffered with 20 mM 2-[4-(2-hydroxyethyl)-1-piperaziny]ethanesulfonic acid (HEPES), pH 7.35 (EMEM-HEPES) and were preincubated for 10 min. The cells were further incubated with drugs for additional 10 min. The medium was acidified to pH 4.0 by addition of 1 N HCl, and PGE₂ was extracted twice with ethyl acetate. After the ethyl acetate was evaporated under a stream of N₂ gas, the sample was dissolved in 10 mM Tris–HCl (pH 7.6). PGE₂ was determined by radioimmunoassay, as previously described.

Measurement of Intracellular Free Ca²⁺ Concentration with Fura 2 Intracellular free Ca²⁺ concentration ([Ca²⁺]ᵢ) was measured as described previously. C6 rat glioma cells
cultured on a 150 mm dish were washed three times with a modified Tyrode solution (composition, mm: NaCl 137, KCl 2.7, MgCl2 1.0, CaCl2 0.18, glucose 5.6, HEPES 10, pH 7.4). The cells were separated from the dish by treatment with 0.1% collagenase and 1.0% bovine serum albumin (BSA) in 10 ml of the modified Tyrode solution for 15 min at 37°C, and were collected into a 50 ml tube followed by centrifugation at 250 g for 1 min. After washing once with 10 ml of the modified Tyrode solution, the cells (1–5×10⁶/ml) were treated with 1 μM fura 2-AM for 15 min and washed twice with modified Tyrode solution. The cells were suspended at 1–5×10⁶/ml, and 1–2 ml of the cell suspension was used for fura 2 assay. Fluorescence of fura 2 at 510 nm by excitation waves at 340 and 380 nm was monitored simultaneously by a spectrofluorometer (Hitachi, F-2000). The ratio of fluorescence at 510 nm by the excitation wave of 340 nm to that of 380 nm was calculated as relative [Ca²⁺].

Data Analysis The statistical significance of the difference between values obtained was determined by Student’s t-test with p<0.05 being regarded as indicative of significance.

RESULTS AND DISCUSSION

Saikosaponin a, b₁, b₂, c, and d are derived from Bupleuri Radix, having chemical structures shown in Fig. 1A. The effects of these drugs on PGE₂ release were examined in C6 rat glioma cells (Fig. 1B). Saikosaponin a, b₁ and d (50 μM) potently stimulated PGE₂ release, while saikosaponin b₂ and c (50 μM) stimulated PGE₂ release to a small extent. The EC₅₀ values were 15.0, 14.4, >50.0, >50.0 and 11.0 μM for saikosaponin a, b₁, b₂, c, and d, respectively. The results are consistent with the report by Ohuchi et al.²²) that saikosaponins stimulate PGE₂ release from macrophages. Since saikosaponin b₁ at 50 μM caused PGE₂ release with the greatest potency among the examined saikosaponins, saikosaponin b₁ was employed for further analysis. Saikosaponin b₁ caused [Ca²⁺]⁺ elevation, which was inhibited by removing extracellular Ca²⁺ with EGTA (Fig. 2). The elevation was not transient, but irreversible. Thus, it is assumed that saikosaponin b₁ may cause the plasma membranes of cells to be permeable to Ca²⁺, and it stimulates PGE₂ release from C6 rat glioma cells mediated via [Ca²⁺]⁺ elevation.

Baicalein is a flavonoid derived from Scutellariae Radix. We investigated whether baicaelin had any antagonistic activity to the PGE₂ release induced by saikosaponin b₁. The saikosaponin b₁-induced PGE₂ release was suppressed by baicaelin in a concentration-dependent manner (Fig. 3). It has been shown that baicaelin inhibits PGE₂ release induced by A23187, a Ca²⁺ ionophore, in C6 rat glioma cells mediated via inhibition of the mitogen-activated protein kinase (MAPK) cascade and cytosolic phospholipase A₂.³⁰) Therefore, it is hypothesized that baicalein antagonizes saikosaponin-induced PGE₂ release with a similar mechanism to the case in A23187-induced PGE₂ release.

In conclusion, saikosaponins, constituents of Bupleuri Radix cause PGE₂ release from C6 rat glioma cells mediated via [Ca²⁺]⁺ elevation. Saikosaponin b₁-induced PGE₂ release was functionally inhibited by baicaelin, a constituent of Scutellariae Radix. The analysis of drug interaction may provide helpful information for interpreting the pharmacological

![Fig. 1. Chemical Structures of Saikosaponin a, b₁, b₂, c, and d, and Their Effects on PGE₂ Release](image)

(A) Chemical structures of saikosaponin a, b₁, b₂, c, and d. (B) The C6 rat glioma cells were incubated with saikosaponin a, b₁, b₂, c, d (each 50 μM) for 10 min. Released PGE₂ in the medium was determined by radioimmunoassay. Each column represents the mean±S.E.M. of three determinations. * Significant difference from control (cont) (p<0.05).

![Table 1. EC₅₀ Values for Saikosaponins in PGE₂ Release from C6 Rat Glioma Cells](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saikosaponin a</td>
<td>15.0±2.02</td>
</tr>
<tr>
<td>Saikosaponin b₁</td>
<td>14.4±2.18</td>
</tr>
<tr>
<td>Saikosaponin b₂</td>
<td>&gt;50.0</td>
</tr>
<tr>
<td>Saikosaponin c</td>
<td>&gt;50.0</td>
</tr>
<tr>
<td>Saikosaponin d</td>
<td>11.0±2.27</td>
</tr>
</tbody>
</table>

Table 1. EC₅₀ Values for Saikosaponins in PGE₂ Release from C6 Rat Glioma Cells

Data were calculated from the concentration-response curve of three determinations.

![Fig. 2. Effect of Saikosaponin b₁ on [Ca²⁺]⁺ in C6 Rat Glioma Cells](image)

[Ca²⁺]⁺ was monitored by the ratio of fluorescence intensity at 510 nm activated by 340 and 380 nm in fura 2-loaded C6 rat glioma cells. (A) Effect of saikosaponin b₁ (50 μM) on [Ca²⁺]⁺ in the presence of external Ca²⁺. (B) Pretreatment with 4 mM EGTA suppressed saikosaponin b₁ (50 μM)-induced [Ca²⁺]⁺ elevation, and addition of 6 mM Ca²⁺ after saikosaponin b₁ caused [Ca²⁺]⁺ elevation.
Fig. 3. Interaction of Saikosaponin b1 with Baicalin in PGE\textsubscript{2} Release from C6 Rat Gloma Cells

The cells were incubated with saikosaponin b1 (b1, 50 \( \mu \)M), baicalin (BL, 50 \( \mu \)M) or saikosaponin b1 (50 \( \mu \)M) plus the indicated concentrations of baicalin (10, 25, 35 and 50 \( \mu \)M) for 10 min. Released PGE\textsubscript{2} in the medium was determined by radioimmunoassay. Each column represents the mean \( \pm \) S.E.M. of three determinations. * Significant difference from saikosaponin b1 alone (\( p < 0.05 \)).

effects of crude drugs or Kampo medicines.

Acknowledgement This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES