Anti-diarrheal effect of *Scutellaria baicalensis* is associated with suppression of smooth muscle in the rat colon

HYUN JU KIM¹, JUN-HO LA², HEE MAN KIM³,⁴, IL-SUK YANG¹ and TAE SIK SUNG⁴

¹Department of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea; ²Department of Neuroscience, Cell Biology and Anatomy, University of Texas Medical Branch, Galveston, TX 77555, USA; ³Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju 26426, Republic of Korea; ⁴Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, NV 89557-0357, USA

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**Abstract.** *Scutellaria baicalensis* (*S. baicalensis*) has been used to manage diarrhea, and its anti-inflammatory effects are responsible for anti-diarrheal effects. However, there are no data concerning its direct effect on colonic motility. Therefore, the effects of the major components of *S. baicalensis* (baicalin, baicalein and wogonin) on colonic motility were investigated. A segment of the distal colon of rats was placed in Krebs solution to monitor spontaneous giant contractions (GCs). Changes in GCs were recorded after applying baicalin, baicalein or wogonin. After pretreatment with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), 1H-(1,2,4)-oxadiazolo (4,2-a) quinoxalin-1-one (ODQ), tetradotoxin, w-conotoxin, apamin, and iberiotoxin, changes in GCs by wogonin were recorded and analyzed. The segment of the distal colon showed spontaneous GCs at a mean amplitude of 3.7±0.3 g with a frequency of 0.8±0.1/min. Baicalin, baicalein, and wogonin reduced both the amplitude and the frequency of GCs in a dose-dependent manner. Wogonin had the most potent inhibitory effect on GCs (IC₅₀ was 14.6 µM in amplitude and 14.2 µM in frequency). Wogonin-induced GC reduction was not significantly affected by the inhibition of nitric oxide/cGMP pathways with L-NAME and ODQ. Blocking the enteric neurotransmission with tetradotoxin and w-conotoxin was ineffective on the wogonin-induced reduction of GCs. Ca²⁺-activated K⁺ (*KᵥCa*) channel blockers (apamin and iberiotoxin) significantly attenuated the inhibitory effects of wogonin on GCs (P<0.01). Wogonin was effective in inhibiting colonic motility, probably through the opening of *KᵥCa* channels located in the smooth muscle apparatus. These findings suggest that wogonin may be a candidate drug for the management of dysmotility-related diarrhea.

**Introduction**

Diarrhea is categorized into four types based on its mechanism of action: Osmotic diarrhea, secretory diarrhea, inflammatory and infectious diarrhea, and diarrhea related to motility disorders. Clinically, diarrhea-predominant irritable bowel syndrome and functional diarrhea are associated with alterations in intestinal motility; therefore, some anti-diarrheal agents decreasing intestinal transit, such as opioids and 5-HT₃ antagonist, have been used to manage these diseases (1).

*Scutellaria baicalensis* is widely used as a herbal medicine in East Asia and Europe for gastrointestinal tract (GI) disorders including dysentery, diarrhea, and abdominal pain (2). Experimentally, it has anti-diarrheal effect on irrinotecan-induced diarrhea in rats (3). Clinically, it demonstrated an improvement in the diarrhea toxicity grade and reduction of frequency of severe diarrhea in lung cancer patients with irrinotecan-induced diarrhea (4). Baicalin, a component of *S. baicalensis* has anti-diarrheal action through its anti-inflammatory effects inhibiting cyclooxygenase-2 (COX-2) activity and colonic prostaglandin E₂ (PGE₂) (5,6).

However, it is still unknown whether *S. baicalensis* directly modulates bowel motility.

Bowel motility is the result of a coordinated contraction and relaxation of intestinal smooth muscles, which is regulated by various neurotransmitters. The neuroeffector apparatus in the GI muscles is conceptualized as the SIP syncytium composed of electrically coupled smooth muscle cells (SMC), interstitial cells of Cajal (ICC), and platelet-derived growth factor receptor α positive (PDGFRα⁺) cells. Various receptors and ion channels are expressed in SIP cells, and conductance changes in any type of SIP cells affect the integrated excitability of muscle layers and responses to neurotransmitters (7).

We hypothesized that if the components of *S. baicalensis* could alter the bowel motility, it is conceivable that they affect the neuromuscular transmission and/or the excitability of SIP cells. Therefore, we examined the effect of the major components of *S. baicalensis* on colonic motility, and investigated...
the mechanism of action of the components on smooth muscle of rat colon.

Materials and methods

Animal and tissue preparation. Male Sprague-Dawley rats (250–300 g), provided ad libitum standard chow and water, were used for this study. The rats were housed in stainless steel hanging cages in a colony room maintained under a 12 h light/dark cycle with a room temperature of 21-23°C and humidity of 65-70%. Their care and handling were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Use and Care Committee at the Seoul National University (Seoul, Korea; approval no. SNU-101872-04). Rats were anesthetized with isoflurane followed by cervical dislocation. From these rats, 2.5-3 cm distal colons were removed and flushed clean with Krebs solution. The colonic segments were immediately placed in a 20 ml organ bath containing Krebs solution bubbled with a mixture of 5% CO₂-95% O₂ (pH 7.4) at 37°C. The preparations were given a minimum of 60-90 min to equilibrate before commencing the experiments. During this period, spontaneous rhythmic giant contractions (GCs) were developed and stabilized.

Measurement of colonic motility. The distal end of the colonic segment was tied to a fixed mount, and the ligated proximal end was secured with silk thread to an isometric force displacement transducer (FT-03; Grass-Telefactor; Astro Med, Inc., Slough, UK). Changes in mechanical signals were detected as isometric tension and recorded as an index of the longitudinal muscle response. After the equilibrium period, spontaneous GCs were recorded. The components of *S. baicalensis* and chemicals were added to the organ bath, after which spontaneous GCs were recorded.

Solutions and chemicals. Krebs solution contained (mM) 10.10 glucose, 115.48 NaCl, 21.90 NaHCO₃, 4.61 KCl, 1.14 NaH₂PO₄, 2.5 CaCl₂, and 1.16 MgSO₄. The following chemicals were used: N’’-nitro-L-arginine methyl ester hydrochloride (L-NAME; 300 µM), 1H-(1,2,4)-oxadiazolo (4,2-a) quinoxalin-1-one (ODQ; 10 µM), tetrodotoxin (TTX; 1 µM), ω-conotoxin (300 nM), apamin (100 nM), and iberiotoxin (100 nM). Each drug was administered 15 min before adding wogonin. Concentrated stock solutions of L-NAME, TTX, ω-conotoxin, apamin, iberiotoxin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) were prepared by dissolving the chemicals in distilled water. ODQ (Sigma-Aldrich; Merck KGaA) was dissolved in dimethyl sulfoxide (DMSO). Baicalin, baicalein and wogonin (Sigma-Aldrich; Merck KGaA) were prepared in 0.2 N NaOH. When DMSO was previously used as a solvent for concentrated stock solutions of baicalin, baicalein, and wogonin, they recrystallized at high doses in Krebs solution and we were unable to measure the exact working concentration. Therefore, in this study, 0.2 N NaOH was used as the solvent to overcome the problem. The dose of baicalin, baicalein, and wogonin was chosen between subminimal and submaximal inhibitory effect of drugs. Even though 500 µM was not submaximal dose of baicalin, highest dose of baicalin was determined at 500 µM due to be recrystallized at over 500 µM.

TTX was used to block the enteric impulse in the rat colon smooth muscle. ω-conotoxin was used to block the neurotransmitter release from nerve terminals by blocking the N-type Ca²⁺ channels (8). Apamin was used to block a small-conductance Ca²⁺-activated K⁺ (SK) channel, which are located in PGD2Rα cells dominantly in the smooth muscle apparatus. Iberiotoxin was used to block a large-conductance Ca²⁺-activated K⁺ (BK) channel, expressed in the SMCs.

Analysis of data. The recorded amplitude and frequency of GCs were analyzed. Two segments from one mouse colon were prepared because some of pretreated drugs were not washed completely. Therefore, we separately analyzed each dataset. To measure the effects of the drug on GCs, each experimental measurement was matched with a control measurement in the same tissue. The amplitude and frequency of GCs in the control state were considered 100% and the parameters of GCs in the presence of baicalin, baicalein, and wogonin were expressed as percentage of the control. For the fitting of the concentration response curves of the *S. baicalensis* components, the following logistical function was used; 

$$Y = 100/(1 + 10^{(IC_{50} - C)/h})$$

where Y is the normalized (%) GC amplitude or frequency at a given concentration (C) of each substance, and h is the slope factor of the curve. IC₅₀ was defined as the concentration of the substance at which Y was inhibited to 50% of the GC amplitude or frequency in the control state. Data were expressed as means ± SEM of n, the number of tissues. Statistical analysis was primarily performed by 2-way repeated measures ANOVA followed by Holm-Sidak multiple comparison test. P<0.05 was considered significant differences.

Results

Inhibitory effect of baicalin, baicalein, and wogonin on GCs in the rat colon. Spontaneous GCs occurred at a mean amplitude of 3.7±0.3 g with a frequency of 0.8±0.1/min in the distal colon of rats (n=38). As shown in Fig. 1, three major components of *S. baicalensis*, namely baicalin (n=14), baicalein (n=12) and wogonin (n=12), reduced the amplitude and frequency of GCs in a dose-dependent manner. After washing off the drugs, the GCs showed complete recovery. The IC₅₀ value of baicalin was 545.4 µM in amplitude and 463.3 µM in frequency. The IC₅₀ value of baicalein was 112.9 µM in amplitude and 123.9 µM in frequency. The IC₅₀ value of wogonin was 14.6 µM in amplitude and 14.2 µM in frequency (Table I). These results show that wogonin is the most effective agent among the three substances in inhibiting GCs in the distal colon of rats.

No effect of nitricergic inhibitory pathway on the wogonin-induced reduction of GCs. In order to evaluate whether wogonin-induced reduction of GCs was mediated by nitric oxide (NO), the NO synthesis inhibitor, L-NAME (300 µM), was used for pre-treatment prior to applying increasing concentrations of wogonin. When comparing the presence and absence of L-NAME, no significant difference in the wogonin-induced reduction of GC amplitude (F₁,ₙ=0.99, P=0.341) and frequency (F₁,ₙ=1.01, P=0.336) (Fig. 2A-C) (n=6-7) could be observed.
Table I. Effects of baicalin, baicalein, and wogonin on the amplitude and frequency of the giant contractions in rat colon (mean ± standard error of the mean).

### A. Amplitude

<table>
<thead>
<tr>
<th>Dose (µM)</th>
<th>Baicalin (%)</th>
<th>Dose (µM)</th>
<th>Baicalein (%)</th>
<th>Dose (µM)</th>
<th>Wogonin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>97.0±3.2</td>
<td>3</td>
<td>96.6±7.5</td>
<td>1</td>
<td>95.4±1.1</td>
</tr>
<tr>
<td>100</td>
<td>94.0±6.1</td>
<td>10</td>
<td>94.6±3.4</td>
<td>3</td>
<td>90.2±3.2</td>
</tr>
<tr>
<td>300</td>
<td>79.0±7.2</td>
<td>30</td>
<td>93.0±6.4</td>
<td>10</td>
<td>72.7±8.0</td>
</tr>
<tr>
<td>500</td>
<td>53.3±12.6</td>
<td>100</td>
<td>64.4±9.1</td>
<td>20</td>
<td>24.7±9.6</td>
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<td></td>
<td></td>
<td>300</td>
<td>0</td>
<td>30</td>
<td>3.8±3.8</td>
</tr>
</tbody>
</table>

P-value: P<0.001

IC$_{50}$ (µM): 545.4

### B. Frequency

<table>
<thead>
<tr>
<th>Dose (µM)</th>
<th>Baicalin (%)</th>
<th>Dose (µM)</th>
<th>Baicalein (%)</th>
<th>Dose (µM)</th>
<th>Wogonin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>93.5±25.2</td>
<td>3</td>
<td>100.3±4.1</td>
<td>1</td>
<td>89.1±5.1</td>
</tr>
<tr>
<td>100</td>
<td>88.2±7.6</td>
<td>10</td>
<td>82.5±5.4</td>
<td>3</td>
<td>86.5±8.6</td>
</tr>
<tr>
<td>300</td>
<td>61.0±6.3</td>
<td>30</td>
<td>80.4±2.9</td>
<td>10</td>
<td>65.5±4.8</td>
</tr>
<tr>
<td>500</td>
<td>46.5±11.7</td>
<td>100</td>
<td>71.3±6.3</td>
<td>20</td>
<td>22.6±8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>0</td>
<td>30</td>
<td>11.7±8.3</td>
</tr>
</tbody>
</table>

P-value: P<0.001

IC$_{50}$ (µM): 463.3

Figure 1. Comparison of the effects of major components of *Scutellaria baicalensis* on the spontaneous GCs. (A) Baicalin, (B) Baicalein and (C) Wogonin reduced the amplitude and frequency of GCs in a dose-dependent manner. Dose-response plots showing inhibitory effect of the three *S. baicalensis* components on the (D) amplitude and (E) frequency of GCs. GCs, giant contractions.
An inhibitor of soluble guanylyl cyclase, ODQ, was used to examine the involvement of cGMP in the wogonin-induced reduction of GCs. The pretreatment with ODQ (10 µM) did not affect the inhibitory effect of wogonin on the amplitude (F_{1,9}=0.485, P=0.504) and the frequency (F_{1,9}=0.259, P=0.623) of GCs (Fig. 2D-F) (n=5-6).

No involvement of enteric neurotransmission in the wogonin-induced reduction of GCs. To evaluate whether wogonin-induced reduction of GCs was dependent on the activation of the enteric nervous system, TTX or ω-conotoxin were pretreated before applying wogonin. In the six distal colon pretreated with TTX (1 µM), the application of wogonin at 1, 3, 10, 20, and 30 µM did not show any significant changes in the amplitude (F_{1,10}=0.181, P=0.679) and frequency (F_{1,9}=0.498, P=0.498), compared to those observed in the control devoid of pretreatment with TTX (Fig. 3A-C).

In the five distal colon pretreated with ω-conotoxin (300 nM), the application of wogonin at 1, 3, 10, 20, and 30 µM did not cause any significant changes in the amplitude (F_{1,8}=0.118, P=0.740) and frequency (F_{1,8}=0.757, P=0.409), compared to those observed in the control devoid of pretreatment with ω-conotoxin (Fig. 3D-F).

Involvement of Ca^{2+}-activated K^+ channels in the wogonin-induced reduction of GCs. The Ca^{2+}-activated K^+ channel (K_{Ca}) channel blockers, apamin and iberiotoxin were used to investigate the participation of K_{Ca} channels in wogonin-induced reduction of GCs. The pretreatment with apamin (100 nM) partially but significantly inhibited the wogonin (20 and 30 µM)-induced reduction of GCs (Tables II and III) (Fig. 4A-C) (n=12-15). Similarly, the pretreatment with iberiotoxin (100 nM) also partially inhibited the wogonin (20 and 30 µM)-induced reduction...
Discussion

This study demonstrates that the three major flavonoids of *S. baicalensis*, baicalin, baicalein and wogonin, inhibit the colonic spontaneous motility in a dose-dependent manner in the rat colon. Of the three substances, wogonin was the most effective in inhibiting GCs. This inhibitory effect of wogonin is associated with K_{Ca} channels in the smooth muscle.

In the aortic and mesenteric arterial smooth muscles, baicalin and baicalein were found to be related to NO-mediated responses and cGMP regulation (9). NO/cGMP pathway is the main physiological mechanism that modulates intestinal motility of both, the duration and amplitude of GCs. NO activates soluble guanylyl cyclase and produces cGMP to relax the smooth muscles by lowering the cytosolic calcium levels and/or by reducing the sensitivity of the contractile elements to calcium (10-13). The association with NO in GCs raised the possibility that wogonin suppresses GCs by increasing the NO release. However, in our study, L-NAME did not affect the wogonin-induced reduction of GCs, which is consistent with a previous report in the aorta (14). Furthermore, a soluble guanylyl cyclase inhibitor, ODQ, also did not affect the inhibitory action of wogonin on GCs. These results indicate that wogonin neither stimulates NO production nor enhances the cGMP-dependent mechanisms in the suppression of GCs.

Other inhibitory enteric neurotransmitters also appear to not mediate the wogonin-induced reduction of GCs. In our study, wogonin still effectively reduced the GCs in the presence of either TTX or ω-conotoxin. These findings suggest that the inhibitory effect of wogonin is not associated with activating
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The inhibitory enteric neural pathways such as NO/cGMP system. Rather, wogonin seems to directly inhibit the smooth muscle contractility to suppress GCs.

Smooth muscle can generate spontaneous GC without neural input, which is termed myogenic contraction. In the tunica muscularis of the colon, SMCs contact ICC and PDGFRα+ cells through a gap junction, resulting in the formation of a SIP syncytium. The SIP syncytium is responsible for the motility of GI muscles classically referred to in the literature as ‘myogenic’ (15). K⁺ channels play an important role in the regulation of contractility, and opening of K⁺ channels contribute to the relaxing effect of various relaxants (16-19). Among the K⁺ channels, KCa channels in the smooth muscles are the important negative feedback elements in limiting the extracellular Ca²⁺ influx-mediated smooth muscle contraction (20, 21). Two types of KCa are identified in smooth muscles: SK channels sensitive to apamin and BK channels sensitive to iberiotoxin (22, 23).

Considering the roles of KCa channels in smooth muscle contractility, it could be hypothesized that KCa channels might participate in the wogonin-induced reduction of GCs. Based on the results of the experiment to test this hypothesis, we

Table II. Effects of SK channel blockers on the wogonin-induced reduction of the GCs (mean ± standard error of the mean).

<table>
<thead>
<tr>
<th>Dose (µM)</th>
<th>Control (%)</th>
<th>Apamin (%)</th>
<th>P-value</th>
<th>Control (%)</th>
<th>Apamin (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.4±1.1</td>
<td>93.2±1.0</td>
<td>0.769</td>
<td>89.1±5.1</td>
<td>90.4±2.1</td>
<td>0.865</td>
</tr>
<tr>
<td>3</td>
<td>90.2±3.2</td>
<td>87.5±1.9</td>
<td>0.915</td>
<td>86.5±8.6</td>
<td>84.9±5.0</td>
<td>0.602</td>
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<tr>
<td>10</td>
<td>72.7±8.0</td>
<td>80.5±3.0</td>
<td>0.26</td>
<td>65.5±4.8</td>
<td>72.5±6.2</td>
<td>0.739</td>
</tr>
<tr>
<td>20</td>
<td>24.7±9.6</td>
<td>59.7±6.8</td>
<td>&lt;0.001b</td>
<td>22.6±8.0</td>
<td>56.5±7.5</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>30</td>
<td>3.8±3.8</td>
<td>56.2±8.0</td>
<td>0.003b</td>
<td>11.7±8.3</td>
<td>45.4±6.4</td>
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</tbody>
</table>

*a,P<0.05, *b,P<0.01 by Holm-Sidak test. GCs, giant contractions.

Table III. Effects of BK channel blockers on the wogonin-induced reduction of the GCs (mean ± standard error of the mean).

<table>
<thead>
<tr>
<th>Dose (µM)</th>
<th>Control (%)</th>
<th>Iberiotoxin (%)</th>
<th>P-value</th>
<th>Control (%)</th>
<th>Iberiotoxin (%)</th>
<th>P-value</th>
</tr>
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<tr>
<td>1</td>
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<td>91.8±0.7</td>
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<td>89.9±7.8</td>
<td>0.787</td>
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<tr>
<td>3</td>
<td>94.0±9.3</td>
<td>95.0±1.5</td>
<td>0.925</td>
<td>88.4±9.3</td>
<td>88.6±9.5</td>
<td>0.989</td>
</tr>
<tr>
<td>10</td>
<td>74.3±6.8</td>
<td>89.8±7.1</td>
<td>0.164</td>
<td>66.9±11.1</td>
<td>77.9±11.9</td>
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</tr>
<tr>
<td>20</td>
<td>33.9±11.5</td>
<td>81.5±10.7</td>
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<td>29.5±8.0</td>
<td>71.7±12.7</td>
<td>0.012</td>
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<td>8.8±8.8</td>
<td>53.5±6.1</td>
<td>0.009</td>
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</table>

*a,P<0.05, *b,P<0.01 by Holm-Sidak test. BK channel, large-conductance Ca²⁺-activated K⁺ channel; GCs, giant contractions.

Table IV. Effects of the combined pre-treatment of SK and BK channel blockers on the wogonin-induced reduction of the giant contractions (mean ± standard error of the mean).

<table>
<thead>
<tr>
<th>Dose (µM)</th>
<th>Control (%)</th>
<th>Apa+Ibe (%)</th>
<th>P-value</th>
<th>Control (%)</th>
<th>Apa+Ibe (%)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>98.7±1.5</td>
<td>97.6±0.5</td>
<td>0.914</td>
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<td>0.941</td>
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<tr>
<td>3</td>
<td>92.4±2.5</td>
<td>98.2±1.0</td>
<td>0.592</td>
<td>89.8±5.8</td>
<td>94.7±5.9</td>
<td>0.823</td>
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<tr>
<td>10</td>
<td>74.0±6.3</td>
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<td>0.053</td>
<td>70.0±7.3</td>
<td>93.2±5.0</td>
<td>0.072</td>
</tr>
<tr>
<td>20</td>
<td>21.8±13.9</td>
<td>88.4±4.5</td>
<td>&lt;0.010a</td>
<td>20.7±11.0</td>
<td>86.6±7.0</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>30</td>
<td>10.1±1.0</td>
<td>82.6±6.1</td>
<td>&lt;0.010a</td>
<td>13.8±9.2</td>
<td>81.0±8.8</td>
<td>&lt;0.001a</td>
</tr>
</tbody>
</table>

*a=P<0.01 by Holm-Sidak test. SK channel, small-conductance Ca²⁺-activated K⁺ channel; BK channel, large-conductance Ca²⁺-activated K⁺ channel; Apa+Ibe; apamin plus iberiotoxin.
Figure 4. Inhibitory effects of SK or BK channel blockers on wogonin-induced reduction of the spontaneous GCs. (A-F) Wogonin-induced reduction of the amplitude and frequency of GCs was partially inhibited in the presence of SK channel blocker, apamin or in the presence of BK channel blocker, iberiotoxin. *P<0.05; **P<0.01 vs. control. GCs, giant contractions; SK channel, small-conductance Ca$^{2+}$-activated K$^+$ channel; BK channel, large-conductance Ca$^{2+}$-activated K$^+$ channel.

Figure 5. Inhibitory effects of both SK and BK channel blockers on wogonin-induced reduction of the spontaneous GCs. (A) Representative trace showing the effect of wogonin on GCs in the presence of SK and BK channel blockers. When the two types of K$_{Ca}$ channels are blocked at the same time, wogonin had no statistically significant inhibitory effect on the (B) amplitude and (C) frequency of GCs. **P<0.01 vs. control. GCs, giant contractions; SK channel, small-conductance Ca$^{2+}$-activated K$^+$ channel; BK channel, large-conductance Ca$^{2+}$-activated K$^+$ channel; K$_{Ca}$ channel, Ca$^{2+}$-activated K$^+$ channel.
found that apamin and iberiotoxin significantly attenuated the inhibitory effect of wogonin on GCs. These data indicate that activation of both SK and BK channels are the pivotal processes in the wogonin-induced reduction of GCs. Previous studies have shown that SK channel expression is dominant in PDGFRα+ cells, although this channel is also present in SMCs (24-28). The current density of SK channels is much higher in PDGFRα+ cells than in the SMCs (29,30). Unlike the SK channel, the BK channel is known to be expressed in SMCs (31-33). Therefore, it is a possibility that wogonin hyperpolarizes PDGFRα+ cells via activation of SK channels to lead to SMC relaxation indirectly, while it hyperpolarizes SMC via activation of BK channels to relax SMC directly. These direct and indirect effects on SMC through different channels result in a reduction of GCs. Wogonin may transiently regulate the intracellular Ca2+ concentration underneath of the membrane through Ca2+ influx or Ca2+ release from the intracellular Ca2+ store in PDGFRα+ cells and SMCs (35). Therefore, reduction of GCs by wogonin might be induced by the opening of Kαs channels directly and/or increase of intracellular Ca2+ concentration underneath of the membrane, and the subsequent opening of KαCa channels, which is closely associated with Ca2+ influx channels and/or Ca2+ release site, indirectly.

The components of S. baicalensis exert various biological actions, especially anti-inflammation in soft tissues (mucosa, submucosa, and skin), but not in smooth muscle (34,35). It has anti-diarrheal effects on irrinotecan-induced diarrhea (3,4). Baicalin has anti-diarrheal action through inhibiting COX-2 activity and PGE2 (5,6). In our study, wogonin, one of components of S. baicalensis, directly inhibited intestinal smooth muscle motility, but not through a mechanism associated with inflammatory pathway. Taken together, this study may provide a completely novel concept of identifying the mechanism of the anti-diarrheal effect of Chinese Skullcap, S. baicalensis. Therefore, the results of this study will serve as a guide to a new therapeutic approach for treatment of acute or chronic diarrhea associated with dysmotility, such as diarrhea-predominant IBS.

In conclusion, our in vitro experiments demonstrated that baikalin, baikaline and wogonin, the components of S. baikalensis, inhibited colonic motility. Wogonin, in particular, directly inhibited the colonic smooth muscle through both SK and BK channels, but not via the activation of inhibitory enteric nerves such as the nitrergic nerves. These findings suggest that wogonin may be a candidate drug for the treatment of increased colon motility diseases, such as diarrhea-predominant IBS.

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Availability of data and materials
The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors’ contributions
HJK, JHL, ISY and TSS conceived the experiments and experimental plan. HJK and TSS performed the experiments. JHL, HMK and TSS collected and analyzed the data. JHL, HMK and TSS wrote the paper. All authors approve the final version of these manuscript and figures.

Ethics approval and consent to participate
Animal care and handling were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Use and Care Committee at the Seoul National University (Seoul, Korea).

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest to disclose with regards to this study.

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