Molecular Mechanism of Anti-Prostate Cancer Activity of Scutellaria Baicalensis Extract

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Abstract: Scutellaria baicalensis is a widely used Chinese herbal medicine historically used in antiinflammatory and anticancer therapy. The goals of the study were to 1) determine its in vitro and in vivo anti-prostate cancer activity, 2) investigate its molecular mechanism directed at cell proliferation control including cyclooxygenase-2 (COX-2) prostaglandin E₂ (PGE₂) and cyclins/cdk pathways, and 3) compare it with those of PC-SPES (PC stands for prostate cancer and spes is Latin for hope), a former herbal mixture for prostate cancer treatment of which S. baicalensis is a major constituent. Two human prostate cancer cell lines (LNCaP, androgen dependent, and PC-3, androgen independent) were assessed for growth inhibition. S. baicalensis exerted dose- and time-dependent increased growth inhibition in both cell lines. However, the PC-3 cells IC₅₀ (50% growth inhibition concentration) were slightly more sensitive than LNCaP cells (IC₅₀ = 0.15 mg/ml), although the former is androgen independent. S. baicalensis was more effective in inhibition of cell growth compared with PC-SPES (IC₅₀ = 0.38 mg/ml for PC-3 cells). Significant reduction of PGE₂ synthesis in both cells after treatment with S. baicalensis resulted from direct inhibition of COX-2 activity rather than COX-2 protein suppression. S. baicalensis also inhibited prostate-specific antigen production in LNCaP cells. Finally, S. baicalensis suppressed expression of cyclin D1 in LNCaP cells, resulting in a G₁ phase arrest, while inhibiting cdk1 expression and kinase activity in PC-3 cells, ultimately leading to a G₂/M cell cycle arrest. Animal studies showed a 50% reduction in tumor volume after a 7-wk treatment period. This study demonstrated that S. baicalensis may be a novel anticancer agent for the treatment of prostate cancer.

Introduction

Prostate cancer is the most frequently diagnosed malignancy in males and the second leading cause of cancer-related death in men after lung cancer. In 2006, the American Cancer Society reported an expected total of 234,460 newly diagnosed prostate cancers and 27,350 prostate cancer–related deaths in the United States (1). Despite significant advances in prostate cancer treatment, the survival rate for advanced stages of this particular cancer has not significantly improved during the past decade (2). The high mortality rate observed in advanced prostate cancer patients is due to loss of androgen dependency for cancer cell growth that results in resistance to androgen ablation therapy (3). Furthermore, standard treatment options for localized prostate cancer (surgical, radiation, and hormonal therapy) are associated with morbidities that often impair patient quality of life, such as urinary incontinence and sexual dysfunction (4). Therefore, prostate cancer patients seek complementary and alternative medicine (CAM) modalities in hopes of halting or slowing disease progression to improve the general quality of life (5). Recent surveys demonstrated that approximately 40% of prostate cancer patients utilize various CAM modalities as a component of therapy (6).

PC-SPES, a proprietary herbal mixture, recently received significant attention in the general public as well as scientific and medical communities. PC-SPES contains Scutellaria baicalensis and seven other herbal extracts (7). PC-SPES has been shown to reduce serum prostate-specific antigen (PSA) levels and proven effective in patients with both androgen-sensitive and androgen-resistant types of prostate cancer (7–9). Importantly, all studies reported a >50% reduction of PSA levels in more than half of patients with advanced androgen-refractory prostate cancer. Unfortunately, PC-SPES was voluntarily removed from the consumer market by its manufacturers due to undeclared substance contamination (that is, warfarin and diethylstilbestrol; http://www.meb.unibonn.de/cancer.gov/CDR0000404384.html). In June 2002, four National Institutes of Health–sponsored PC-SPES–associated studies, one of which included a clinical trial, were placed on hold. Since then, three laboratory studies have resumed their investigations (http://nccam.nih.gov/health/alerts/spes/).

S. baicalensis Georgi, also known as huang qin, or Chinese skullcap, has been used for thousands of years in traditional Chinese medicine as an antiinflammatory, antibacterial, and anticancer remedy to treat respiratory infections, gastrointestinal disorders, hepatitis, and cancer. Our recent
study demonstrated that the raw extract of *S. baicalensis* inhibits cancer cell growth in different cell lines, including prostate, squamous cell, and breast cancers (10,11). We further demonstrated that the *S. baicalensis* extract decreased PGE2 production via the suppression of cyclooxygenase-2 (COX-2) activity, which is often elevated in various types of cancers (10,12,13).

Ample evidence supports involvement of the COX-2 pathway in prostate carcinogenesis via several mechanisms, including stimulation of tumor invasion factors, such as vascular endothelial growth factor (VEGF) expression (14), release of matrix metalloproteinases (15), facilitation of inflammation (16), and possible contribution of the COX-2 product PGE2 to enhanced cell proliferation (16,17). Uncontrolled cell proliferation that leads to cancer formation is caused by disruption of proper cell cycle regulation, a pivotal characteristic of cancer cells. Increased levels of the G0/G1 checkpoint controlling cyclins D1 and E, and their associated protein kinases (cdks), concurrently with decreased levels of cdk inhibitors (Cip/Kip family), were reported in prostate cancer LNCaP cells (18) and in animal models (19).

The purpose of this study is to evaluate *S. baicalensis* as an anti-prostate cancer agent both in vitro (in androgen-dependent and androgen-independent cell lines) and in vivo (in nude mice xenografted with prostate cancer cells) and to explore its ability to affect both COX-2 and cell cycle regulation pathways to further understand the molecular mechanism of its anti-prostate cancer activity. We hope the knowledge gained by our research will significantly contribute to the development of new and effective therapy for this deadly disease.

**Materials and Methods**

**Chemicals and Drugs**

The raw extract of *S. baicalensis* was prepared by boiling the dried root of the plant in water, followed by a spray-drying process of the resulting water extract. The powder form of the extract was obtained from E-Fang Pharmaceutical Company (Guangdong, China) and contains baicalin, wogonoside, and oroxylin A as major components. The extract was dissolved in culture medium to 20 mg/ml, vortexed at room temperature for 1 min, and incubated at 37°C for 1 h while rotating before use. This solution was centrifuged at 5,000 rpm for 10 min to remove any insoluble ingredients. The supernatant was passed through a 0.22-µm filter for sterilization and diluted with culture medium to final concentrations of 0.04–0.8 mg/ml of *S. baicalensis* extract. The quality and authenticity of *S. baicalensis* were assessed using high-performance liquid chromatography (20). For result consistency, the same batch was used throughout the entire study. A 10-nM stock solution of celecoxib [SC-58635; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1]-benzene-sulfonamide, Searle Research and Development, St. Louis, MO], a selective COX-2 inhibitor, was prepared with dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO). PC-SPES (Lot# 5431060) in capsule form (320 mg per capsule) was purchased from Botanic Lab (Brea, CA). Because most published studies on PC-SPES were performed using ethanol as a solvent vehicle to dissolve the extract (21,22), we used both media as well as ethanol to dissolve *S. baicalensis* and PC-SPES to final concentrations of 0.0015–1.5 mg/ml, following the same preparation steps as described previously.

**Cell Lines and Cell Culture**

Two human prostate cancer cell lines, LNCaP (androgen dependent) and PC-3 (androgen independent), were purchased from American Type Culture Collection (Rockville, MD). These two cell lines represent the two major types of prostate cancer: androgen-dependent/sensitive and androgen-independent/insensitive prostate cancer cell populations, the latter more typical of advanced prostate cancer (23). Both cell lines were maintained in RPMI 1640 medium containing 10% GIBCO™ fetal bovine serum (Invitrogen, Carlsbad, CA) at 37°C in a humidified atmosphere of 5% CO2.

**Cell Viability Assay**

The percentage of growth inhibition was determined by employing a 3,4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay to measure viable cells. Cells (4 × 10³ cells per well) were seeded onto a 96-well plate for 24 h, treated with various concentrations of *S. baicalensis*, and incubated for an additional 3 or 5 days at 37°C. Subsequently, 10 µl of MTT at a concentration of 5 mg/ml was added to each well, and cells were incubated for an additional 4–6 h. The supernatant was aspirated, and 100 µl of DMSO was added to the wells to dissolve any precipitate present. The optical density was then measured at a wavelength of 570 nm using an ELX800 plate reader (Bio-Tek Instruments, Winooski, VT).

**Cell Cycle Analysis**

Cells (1.5 × 10⁵ cells per well) were plated onto six-well plates and incubated for 24 h at 37°C. Various concentrations of *S. baicalensis* were added to the wells and incubated for an additional 3 days. Cells were then washed, pelleted, fixed with cold 70% ethyl alcohol for at least 30 min at –20°C, and incubated with 100 µg/ml RNase A and 50 µg/ml propidium iodide in phosphate-buffered saline at room temperature for 30 min. Samples were immediately analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). The percentage of cell cycle distribution was determined using Cell Quest Pro software (Becton Dickinson, Franklin Lakes, NJ).
Quantitation of Prostate-Specific Antigen

LNCaP cells, known to produce high levels of PSA (24), were plated onto a six-well plate at a density of 1.5 × 10^5 cells per well. After a 24-h incubation period, the medium was replaced with fresh medium containing 50% growth inhibition concentration (IC_{50}) doses of S. baicalensis (0.15 mg/ml) or celecoxib (25 µM). After 72 h of treatment, the medium was collected and centrifuged to eliminate detached cells. PSA level in 150 µl supernatant was determined using an AxSYM PSA Kit (Abbott Laboratories, Abbot Park, IL) according to manufacturer’s instructions. PSA is expressed as picograms per 10^6 cells.

PGE2 Enzyme Immunoassay

To quantify the level of PGE2 released into culture media, a competitive PGE2 enzyme immunoassay was performed according to manufacturer’s instructions (Amersham Pharmacia Biotech, Piscataway, NJ). Supernatants of control and S. baicalensis (0.075–0.3 mg/ml)-treated cells were collected, centrifuged at 2,500 rpm for 2 min, and stored at −70°C until further analysis. Aliquots (50 µl) of each sample were assayed in triplicate. Optical density was measured at 450 nm on an ELX800 plate reader (Bio-Tek Instruments). The minimal detectable concentration of PGE2 by the assay is 50 pg/ml. PGE2 is expressed as picograms per 10^6 cells. The effect of celecoxib and S. baicalensis on COX-2 activity was further examined using an intracellular arachidonic acid conversion assay (11,25). After a 24-h incubation of LNCaP or PC-3 cells, the medium was aspirated and replaced with fresh medium containing 0.15 mg/ml S. baicalensis or 25 µM celecoxib. Cells were incubated for additional periods of time, and the medium was aspirated. After washing the cells with medium, fresh medium containing 100 µM arachidonic acid (Cayman Chemical, Ann Arbor, MI) was added and incubated for an additional 30 min. PGE2 in the medium was measured as described previously and expressed as picograms per milliliter of medium.

Western Blot Analysis

To assess dose-dependent protein expression change, LNCaP or PC-3 cells were treated for 72 h with various concentrations of S. baicalensis (0.1–0.8 mg/ml) in a dose-dependency study or with a single concentration of S. baicalensis (0.4 mg/ml) in a time-course study. Cellular proteins were extracted as described previously. One milligram of protein was further incubated with 2 µg of anti-cdk1 antibody (Santa Cruz Biotechnology) at 4°C, followed by incubation with 25 µl (25% agarose) of protein A-agarose beads (Santa Cruz Biotechnology) at 4°C on a rotator overnight. After two sequential wash cycles with a lysis buffer and kinase buffer (30 mM Tris-HCl, 10 mM MgCl2, and 1 mM dithiothreitol), the immunoprecipitate was resuspended in 30 µl kinase buffer containing 4 µg Histone H1 (cdk1 kinase substrate), 50 µM of unlabeled adenosine triphosphate (ATP), and 5 µCi of [γ-32P]ATP at 30°C for 30 min. After the addition of 2X SDS loading buffer and heating at 100°C for 5 min, the reaction products were separated on 12% SDS–polyacrylamide gel electrophoresis and visualized by autoradiography.

Animals and Treatment Protocol

Six-week-old male nude mice (NCR-nu/nu) were purchased from Taconic (Cincinnati, OH). Each animal received a single-bolus subcutaneous injection of 3 × 10^6 PC-3 cells to the right flank. After 1 wk of inoculation to allow for adequate tumor cell engraftment (average tumor mass ≈150 mm^3), the mice were randomized into two experimental groups (n = 5). The treatment group was given a 200-mg/kg dose of S. baicalensis extract dissolved in water by oral gavage once
a day, five times per week for 7 wk. Mice were weighed, and tumor volume was assessed on a weekly basis. Tumor volume was measured by two perpendicular dimensions (long and short) using a caliper and calculated using the formula \((a \times b^2)/2\), where \(a\) is the larger and \(b\) is the smaller dimension of the tumor. Treatment and care of experimental animals were in accordance with the institutional guidelines of the Mount Sinai School of Medicine.

### Results

**Growth Inhibition Effect of *Scutellaria Baicalensis* on Prostate Cancer Cells**

To determine the anti-prostate cancer activity of *S. baicalensis* extract, we performed in vitro growth inhibition assays on LNCaP and PC-3 cells. The rate of inhibition was determined as a percentage of viable treated cells compared with viable control cells. *S. baicalensis* displayed a dose-dependent (Fig. 1A) and time-dependent (Fig. 1B) growth inhibition in both cell lines. Although both LNCaP and PC-3 cells were sensitive to *S. baicalensis*, this effect was not equally observed. The androgen-independent PC-3 cells exhibited a slightly increased sensitivity. IC\(_{50}\) was 0.15 mg/ml for LNCaP cells and 0.1 mg/ml for PC-3 cells after 3 days of incubation with *S. baicalensis*, and this 0.05-mg/ml difference remained after 5 days of treatment (data not shown). We then compared the effect of *S. baicalensis* and PC-SPES on the androgen-independent PC-3 cell line. On a weight-by-weight basis (information on the percentage of *S. baicalensis* in PC-SPES is unavailable), *S. baicalensis* was more effective in growth inhibition (IC\(_{50}\) = 0.16 mg/ml in medium and 0.06 mg/ml in ethanol) than PC-SPES (IC\(_{50}\) = 0.38 mg/ml in medium and 0.1 mg/ml in ethanol), regardless of the solvent vehicle used (Fig. 1C).

Finally, we analyzed the effect of *S. baicalensis* on the cell cycle distribution of both cell lines. Our results markedly differed for LNCaP and PC-3 cells. Compared with controls (Fig. 2A and C), *S. baicalensis* caused a significant G\(_0\)/G\(_1\) phase arrest with a concurrent decrease of the S and G\(_2\)/M phases in LNCaP cells (Fig. 2B). A different effect was observed in PC-3 cells, where *S. baicalensis* caused a significant G\(_2\)/M arrest, concurrently increasing the S phase and decreasing the G\(_0\)/G\(_1\) phase (Fig. 2D).

**Inhibition of PSA Production in LNCaP Cells**

PSA has been recognized as a biomarker for monitoring treatment response to prostate cancer. Therefore, we investigated whether *S. baicalensis* can inhibit PSA production. Our analysis demonstrated a high level of PSA production in LNCaP cells (196 ng per 10\(^6\) cells) (Fig. 3) but not in PC-3 cells (data not shown), which is consistent with a previous report (26). After 72 h of treatment with the IC\(_{50}\) dose of *S. baicalensis* (0.15 mg/ml) and celecoxib (25 \(\mu\)M), PSA levels decreased to 75.5 and 51.2 ng per 10\(^6\) cells, respectively, indicating a significant inhibition of PSA production. These results suggest that *S. baicalensis* suppresses the pathway controlling PSA synthesis, presumably via an androgen receptor. These results support the findings of a recent study that *S. baicalensis* components (baicalein, wogonin, beobaicalein, and skullcapflavone) can suppress PSA production as well as androgen receptor expression in LNCaP cells (27).

**Inhibition of COX-2 Activity and Expression**

Increased levels of COX-2 and its product PGE\(_2\) have been identified in prostate cancer cells. Therefore, we determined the level of PGE\(_2\) in LNCaP and PC-3 cells and the effect of *S. baicalensis* on PGE\(_2\) synthesis. We observed elevated concentrations of PGE\(_2\) in both cell lines (Fig. 4A), which support the findings of previous investigations (28,29).

![Figure 1. Inhibition of LNCaP and PC-3 cell growth by *Scutellaria baicalensis* and PC-SPES. (A) Dose-dependent inhibition of LNCaP and PC-3 cells after treatment with *S. baicalensis* for 72 h, (B) time-dependent growth inhibition of PC-3 and LNCaP cells treated with 0.1 mg/ml of *S. baicalensis*, and (C) comparison of dose-dependent inhibition of the herbal mixture PC-SPES and *S. baicalensis*, its major active component, for 72 h. Results represent mean values ± SD (bars) of three independent experiments performed in triplicate. SB, *S. baicalensis*; M, medium as solvent; E, ethanol as solvent.](image-url)
However, LNCaP cells produced a notably higher level of PGE2 (39.1 pg per 10^6 cells) compared with PC-3 cells (26.9 pg per 10^6 cells) after 12 h of incubation. *Scutellaria baicalensis* significantly inhibited PGE2 production in LNCaP cells starting at a concentration of 0.075 mg/ml and in PC-3 cells starting at a concentration of 0.15 mg/ml. PGE2 production continued to decline in both cell lines as *S. baicalensis* concentrations increased (up to 0.3 mg/ml).

To further investigate the mechanism by which *S. baicalensis* inhibits PGE2 production, we examined its effect on COX-2 enzymatic activity using an intracellular arachidonic acid conversion assay that measures PGE2 production after the addition of exogenous arachidonic acid to the newly replenished medium of treated cells. For comparison, a known COX-2 inhibitor, celecoxib, was included in this assay. By providing exogenous arachidonic acid, a COX-2 substrate, we sought to eliminate the effect of endogenous arachidonic acid release from the cell membrane due to intracellular protein kinase and/or phospholipase A2 activities. Our results demonstrate a significant decrease of PGE2 level in the presence of both *S. baicalensis* and celecoxib at IC50 doses in both PC-3 and LNCaP cells during a 24-h time period (Fig. 4B and C). For each cell line, the pattern of PGE2 inhibition was similar for celecoxib and *S. baicalensis*, although a slight delay of inhibition by *S. baicalensis* in PC-3 cells (2-h lag period for *S. baicalensis* vs. immediate for celecoxib) was observed (Fig. 4C). The level of decreased PGE2 production was similar in both cell lines, that is, a drop of approximately 200 pg/ml (from 1,048 to 853 pg/ml for LNCaP cells and from 482 to 264 pg/ml...
Figure 4. Inhibition of PGE2 synthesis and cyclooxygenase-2 activity by *Scutellaria baicalensis* and celecoxib. (A) Dose-dependent suppression of PGE2 synthesis after 12-h incubation with S. baicalensis. Suppression of arachidonic acid conversion to PGE2 by S. baicalensis (0.15 mg/ml) and celecoxib (25 µM) in (B) LNCaP and (C) PC-3 cells. Results represent mean values ± SD (bars) of three independent experiments performed in triplicate. Data were analyzed using the Student’s t-test. *P < 0.05 and **P < 0.01 compared with the control groups.

for PC-3 cells). However, the inhibitory effect in LNCaP did not occur until 12 h after the addition of arachidonic acid (Fig. 4B). These results strongly support our hypothesis that *S. baicalensis* inhibits PGE2 synthesis through COX-2 activity.

We also investigated the effect of *S. baicalensis* on COX-2 expression using Western blotting (Fig. 5). The effect of *S. baicalensis* treatment on COX-2 expression differed in two cell lines, for both time-course and dose-dependency studies. In PC-3 cells, which had a lower baseline COX-2 expression...
level than LNCaP cells, treatment with *S. baicalensis* induced COX-2 expression in a time- and dose-dependent manner (Fig. 5A and B). In contrast, treatment of LNCaP cells with *S. baicalensis* slightly decreased COX-2 expression (Fig. 5C and D). These results further suggested that decreased PGE_2_ synthesis induced by *S. baicalensis* is likely due to the direct inhibition of enzymatic activity of COX-2 rather than its expression.

**Effect on Cell Cycle–Regulating Molecules**

Cell cycle analysis revealed that *S. baicalensis* treatment induced G_1_ arrest for LNCaP cells and G_2_ for PC-3. Therefore, we further evaluated the effect of *S. baicalensis* on cyclin D1 and cyclin B1 expression, two factors important for cell progression from the G_1_ to S and the G_2_ to M phases, respectively (Fig. 6). When LNCaP cells were treated with various concentrations of *S. baicalensis*, cyclin D1 expression decreased in a dose-dependent manner (Fig. 6A). We also observed a steady decrease in cyclin D1 expression in the course of 72 h, which at the end dropped to undetectable levels (data not shown). No change in cyclin D1 expression was observed in PC-3 cells, as expected (Fig. 6C). These results indicate that *S. baicalensis* suppresses the expression of cyclin D1 in LNCaP cells, resulting in G_1_-phase arrest.

When cyclin B1 was analyzed, its level of expression did not change in both cell lines (Fig. 6B and D), although a marked G_2_/M-phase arrest in PC-3 cells was observed after treatment with *S. baicalensis*. Therefore, we further examined the expression of cdk1 protein kinase (p34Cdc2), the catalytic subunit of the cyclin B1/cdk1 complex. Our results showed that treatment with different doses of *S. baicalensis* for 72 h resulted in a slight decrease of cdk1 expression in PC-3 cells (Fig. 7A). Time-course studies confirmed the efficacy of this dose after 72 h of treatment (Fig. 7B). As expected, no change in cdk1 expression in LNCaP cells was observed after treatment with *S. baicalensis* (Fig. 7E). We then determined the effect of *S. baicalensis* on cdk1 kinase activity by analyzing the level of histone H1 phosphorylation, the substrate of the cyclin B1/cdk1 complex, using a kinase activity assay. We observed both a dose- and time-dependent decrease of cdk1 kinase activity in PC-3 cells after treatment with *S. baicalensis* (Fig. 7C and D). These results indicate that *S. baicalensis* inhibits primarily cdk1 kinase activity in PC-3 cells leading to the G_2_/M-phase arrest.

![Figure 6. Effect of *Scutellaria baicalensis* on cyclin D1 and cyclin B1 protein expression. LNCaP and PC-3 cells were treated with various concentrations of *S. baicalensis* for 72 h, and the expression levels of cyclin D1 (A and C) and cyclin B1 (B and D) were determined by Western blot. β-Actin was used as an internal control. Three independent experiments were performed, each with similar results.](image-url)
Figure 7. Effect of *Scutellaria baicalensis* on cdk1 expression and its kinase activity. cdk1 expression in (A) PC-3 and (E) LNCaP cells by various concentrations of *S. baicalensis* for 72 h. (B) Time-dependent inhibition of cdk1 expression by *S. baicalensis* (0.4 mg/ml) in PC-3 cells. (C) Dose-dependent and (D) time-dependent suppression of cdk1 activity by *S. baicalensis* (0.4 mg/ml) for time course in PC-3 cells. β-Actin was used as an internal control. Three independent experiments were performed, each with similar results.

**Inhibition of Tumor Growth in PC-3–Xenografted Nude Mice**

To confirm the anticancer activity of *S. baicalensis* in vivo, nude mice were subcutaneously inoculated with PC-3 cells and treated with *S. baicalensis* by oral gavage at a dose of 200 mg/kg of body weight once a day, five times per week for 7 wk. Our results demonstrated a significant inhibition of tumor growth in the treated mice compared with the untreated animals (Fig. 8). Average tumor volume at Week 8 was

Figure 8. Inhibition of tumor growth in nude mice by *Scutellaria baicalensis*. One week after subcutaneous inoculation with PC-3 cells, mice were randomly assigned into two groups, five animals per group. In the treatment group, *S. baicalensis* was given orally at a dose of 200 mg/kg of body weight once a day, five times per week for 7 wk. (A) The tumor volume of each animal was measured every week, and the mean tumor volume ± SD (bars) is graphed against time (week). The arrow represents the starting week of *S. baicalensis* treatment. Data points represent mean tumor volume ± SD (bars) of mice. A comparison between control and treated tumor volumes at the 8th wk was performed using the Student’s *t*-test.* *P* < 0.05 compared with the control group. (B) The body weight of each animal was also measured weekly and graphed against time (week).
S. baicalensis has been shown to have a broad spectrum of biological activities, including anti-inflammatory (30) and anticancer (10). The present study confirmed that S. baicalensis has a strong dose-dependent inhibitory activity against both androgen-dependent (LNCaP) and androgen-independent (PC-3) types of prostate cancer, with established IC_{50} doses at 0.15 mg/ml for LNCaP cells and 0.1 mg/ml for PC-3 cells (Fig. 1A). The ability to inhibit prostate cancer growth was further confirmed in our animal study (Fig. 8). The ability of S. baicalensis extract to inhibit both androgen-dependent and androgen-independent prostate cancer cells is clinically relevant and important. It is clear that prostate cancers frequently develop resistance to a conventional therapeutic regimen including androgen ablation. Once the prostate cancer cells become androgen independent, clinical failure often occurs, with a median survival of 9–12 mo. Therefore, S. baicalensis may be effective for the treatment of both androgen-independent and androgen-dependent prostate cancers. These promising results support the future clinical trial of this herbal extract to determine its efficacy as an anti-prostate cancer agent.

It is worthy to note S. baicalensis is more effective than PC-SPES (Fig. 1C), a popular herbal mixture for the treatment of prostate cancer that contains eight herbs including S. baicalensis. In support of our finding, a recent study demonstrated that S. baicalensis is one of the herbal components that inhibit prostate cancer cell growth (LNCaP) in vitro, and S. baicalensis is the only herbal component with antiandrogenic activity (27). These results also demonstrated that S. baicalensis may be a viable alternative for PC-SPES, given the quality-control and adulteration concerns. The use of a single herb reduces the side effects and possible antagonistic interactions of multiple herbal mixtures and eliminates the quality-control issues, such as contamination, adulteration, and/or poor quality, that are associated with multiple herbal mixtures (20,31).

The anticancer activity of S. baicalensis may be derived from its ability to inhibit cancer cell proliferation. We did not observe increased apoptosis in both cell lines after treatment with S. baicalensis (data not shown). However, S. baicalensis caused arrest at different cell cycle phases in these two cell lines, that is, G1 arrest for androgen-dependent LNCaP cells and G2 arrest for androgen-independent PC-3 cells (Fig. 2). At the molecular level, we demonstrated that S. baicalensis reduced expression of cyclin D1 in androgen-dependent LNCaP cells, which is responsible for the G1-to-S-phase transition of the cell cycle (Fig. 6A). In androgen-independent PC-3 cells, a significant change in cyclin D1 and B1 expression was not observed; however, a significant decrease in cdk1 kinase expression and activity after treatment with S. baicalensis was noted (Figs. 6 and 7). These findings are consistent with a recent study showing that the major components of S. baicalensis (baicalein, wogonin, beobaicalein, and skullcapflavone) induce G1 arrest for LNCaP and G2 arrest for PC-3 cells (27). However, it is unclear why S. baicalensis differentially affects these two prostate cancer cell lines. This may be due to different regulatory mechanisms of cell proliferation between these two cell lines. It has been shown that LNCaP and PC-3 cells exhibit significant difference in their gene expression patterns, particularly the androgen-related signal transduction pathways. Karan et al., using a microarray method, identified 34 up-regulated and 8 down-regulated genes in androgen-dependent cells compared with androgen-independent cells (32). Therefore, although S. baicalensis acts on the same signal transduction pathway (that is, COX-2), the possibility exists that the downstream effect of COX-2 is different in these two cell lines (that is, G1 vs. G2 arrest).

The ability of S. baicalensis to affect cell cycle–regulating molecules is an important finding because elevated levels of cyclin D1 promote prostate cancer development by reducing its dependence on androgen stimulation (18). Additionally, there is evidence that androgens may induce G1/S transition and thus contribute to cancer cell proliferation (19). Increased expression of G1-phase kinases cdk2 and cdk4 and increased activity of cdk2 kinase were observed as a result of androgen stimulation as well as down-regulation of cdk4 inhibitor p16 (33) and cdk2 inhibitor p21 (34). Changes in the expression of cell cycle–regulating genes, such as cyclins A and D, cdk2, and p21, were also observed in PC-SPES–treated prostate cancer cells (35). Due to the critical role of these cell cycle–regulating molecules in cancer development, these molecules have become important targets for drug development. For example, flavopiridol, a semisynthetic flavonoid derived from the bark of the Dysosyllum binectariferum plant indigenous to India, specifically targets cdk5s to block cell cycle progression at the G1/S and G2/M checkpoints (36). S. baicalensis may prove to be a good candidate for this class of agent.

Although it is possible that S. baicalensis acts directly on cell cycle–regulating molecules, such as cyclin D1 and cdk1, it is also possible that S. baicalensis may act on other upstream cell cycle–regulating molecules. It has been shown that COX-2 and its product PGE2 are important molecules in regulating prostate cancer cell development and progression, particularly during the early stages (37). For example, in PC-3 cells, PGE2 induced the immediate-early gene c-fos via the protein kinase A pathway by activating prostaglandin EP-2/4 receptors, whereas COX-2 stimulates VEGF expression, resulting in increased proliferation (38,39). Furthermore, COX-2–specific inhibitors celecoxib and nimesulide dramatically reduced the expression of androgen-inducible genes, such as PSA, as well as androgen receptor expression via suppression of their promoter activity (40). Our results showed that both prostate cancer cell lines had increased COX-2 expression.
(Fig. 5) and increased PGE2 production (Fig. 4) (more so in androgen-sensitive LNCaP cells), consistent with previous reports (28,29). S. baicalensis suppressed PGE2 synthesis in both cell lines (Fig. 4A), and the pattern of PGE2 inhibition was similar for both celecoxib, a COX-2–specific inhibitor, and S. baicalensis (Fig. 4C), indicating that S. baicalensis acts on COX-2 enzymatic activity directly. However, it is unclear why a delay of inhibition of PGE2 synthesis in LNCaP cells was observed (Fig. 4B). Analysis of COX-2 expression in both cell lines revealed either slightly decreased (LNCaP) or increased (PC-3) COX-2 expression (Fig. 5), further supporting the notion that decreased PGE2 synthesis resulted from direct inhibition of COX-2 activity. Although the crosstalk between COX-2/PGE2 and cell cycle regulation (that is, cyclin D1 and cdk1) remains unclear, it is plausible that COX-2 and/or PGE2 acts on cell cycle regulation through intermediate kinase pathways such as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) (41). It is interesting to note that both S. baicalensis and celecoxib can inhibit PSA production in LNCaP cells (Fig. 3), suggesting that COX-2 also interacts with the androgen signal transduction pathway. In a phase II clinical trial, 92% of prostate cancer patients who received celecoxib treatment for 3 mo showed a significant reduction of PSA level, further confirming the regulatory relationship of COX-2 and PSA (42). It is worthy to note that, although S. baicalensis inhibits androgen receptor expression (27), the androgen signal pathway alone cannot explain its anti-prostate cancer activity because both androgen-dependent and androgen-independent prostate cancer cells are sensitive to S. baicalensis.

In summary, this study, together with others (27), demonstrated that S. baicalensis, the major component of the mixed herbal formula PC-SPES, is responsible for its anti-prostate cancer activity. More importantly, it inhibits androgen-independent prostate cancer cells (PC-3) more strongly than it inhibits androgen-dependent cells (LNCaP), which could be a viable alternative to prostate cancer patients who fail to respond to conventional hormone-based treatment. Inhibition of PSA production in LNCaP cells not only proves that S. baicalensis can inhibit the androgen-dependent signal transduction pathway but also suggests that PSA can be used as an intermediate marker for monitoring the response to S. baicalensis treatment for future clinical trials. Mechanistically, S. baicalensis targets multiple cell proliferation pathways, including COX-2/PGE2, cyclin D1, and cdk1, resulting in the suppression of prostate cancer growth at the G1 or G2 phases. Future clinical study is necessary to determine its efficacy in prostate cancer patients.

Acknowledgments and Notes

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