Research Article

Anti-Bladder-Tumor Effect of Baicalein from *Scutellaria baicalensis* Georgi and Its Application *In Vivo*

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Some phytochemicals with the characteristics of cytotoxicity and/or antimetastasis have generated intense interest among the anticancer studies. In this study, a natural flavonoid baicalein was evaluated in bladder cancer *in vitro* and *in vivo*. Baicalein inhibits 5637 cell proliferation. It arrests cells in G1 phase at $100\,\mu$M and in S phase below $75\,\mu$M. The protein expression of cyclin B1 and cyclin D1 is reduced by baicalein. Baicalein-induced p-ERK plays a minor role in cyclin B1 reduction. Baicalein-inhibited p65NF-κB results in reduction of cell growth. Baicalein-induced pGSK(ser9) has a little effect in increasing cyclin B1/D1 expression instead. The translation inhibitor cycloheximide blocks baicalein-reduced cyclin B1, suggesting that the reduction is caused by protein synthesis inhibition. On the other hand, neither cycloheximide nor proteasome inhibitor MG132 completely blocks baicalein-reduced cyclin D1, suggesting that baicalein reduces cyclin D1 through protein synthesis inhibition and proteasomal degradation activation. In addition, baicalein also inhibits cell invasion by inhibiting MMP-2 and MMP-9 mRNA expression and activity. In mouse orthotopic bladder tumor model, baicalein slightly reduces tumor size but with some hepatic toxicity. In summary, these results demonstrate the anti-bladder-tumor properties of the natural compound baicalein which shows a slight anti-bladder-tumor effect *in vivo*.

1. Introduction

Bladder cancer is the seventh most common type of cancer in worldwide man [1] and fourth in man of United States [2]. More than 90% of bladder cancers are transitional cell carcinoma (TCC), and approximately 80% of TCC belong to noninvasive papillary carcinoma that is a low-grade intraurothelial neoplasia with high recurrence. The other 20% of TCCs initiated from carcinoma in situ are at a high risk of processing to muscle invasive disease with a substantial risk for the development of distant metastasis [3, 4]. More than 10% of the low-grade papillary tumors eventually progress to high-grade muscle invasive bladder tumors. Most of the deaths from bladder cancer patients are due to invasive cancer metastasis [5], which has been a leading problem in the cancer therapy field. Multiple drugs chemotherapy has been applied for the therapy of metastatic bladder

cancer; however, the adverse effect and resistance usually limit its clinical result. Therefore, some phytochemicals with the characteristics of cytotoxicity and/or antimetastasis have generated intense interest among the anticancer studies.

Baicalein, one of four major flavonoids existed in the root of *Scutellaria baicalensis* Georgi, has excellent antioxidant and anti-inflammatory activities [6, 7]. In traditional Chinese herb medicine, the root of *Scutellaria baicalensis* Georgi was usually gathered before Tomb-Sweeping Day and decocted for the purpose of "cleansing heart" and "removing toxins," for example, cough with yellow sputum, jaundice, swelling and pain of eye, and so on. Wogonin, another one of the major flavonoids in the root of *Scutellaria baicalensis* Georgi, has been reported to reduce inflammatory cyclooxygenase-2 expression by c-Jun inhibition [8]. In addition to the anti-inflammatory effect of wogonin, baicalein has been reported to apply in cancer therapy by its cytotoxicity [9–11] and its

anti-metastasis activity [12–14] recently. In human pancreatic cancer cells, 15~50 μM baicalein induces apoptotic cell death through downregulation of an antiapoptotic protein Mcl-1 [11]. In human bladder cancer cells, 60~80 μM baicalein retards cell growth by inhibiting CDC2 kinase activity [9]. Sixty μM baicalein also induces bladder cancer cells death, but baicalein-induced p-Akt and γ-H2AX expression plays a protective role against cell death [10]. Moreover, 10~50 μM baicalein inhibits cell migration and invasion through inhibiting MMP-2/9 activity in human hepatoma cells [12] and human breast cancer cells [13]. In human skin carcinoma, 40 μM baicalein inhibits cell invasion through inhibiting an anchor protein Ezrin expression [14]. Recently, baicalein is proven to be genotoxic without producing chromosomal alterations and mutagenesis which results in the severe side effect in cancer chemotherapy [15]. According to the above data, baicalein is a candidate worth development in anticancer therapy.

In this study, the anticancer effect of baicalein was analyzed in bladder cancer cells *in vitro* and in an orthotopic bladder tumor model *in vivo*. *In vitro*, the correlation of baicalein-induced change in Akt, ERK, p38, and p65NF-κB pathways and cell viability was analyzed. *In vivo*, the antitumor effect and renal and hepatic toxicities were evaluated.

2. Materials and Methods

2.1. Cell Culture and Drug Preparation. Human bladder papillary transitional cell carcinoma 5637 cells were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Mouse bladder carcinoma MB49 cells were kindly provided by Dr. Timonthy L. Ratliff (Purdue Cancer Center, *West Lafayette, IN, USA).* 5637 and MB49 cells were maintained in RPMI 1640 medium supplied with 10% fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin. Cells were incubated in a CO_2 incubator at 37°C, with 5% CO_2 and 95% filtered air. Baicalein was isolated from the root of *Scutellaria baicalensis* Georgi, identified [16] and dissolved in DMSO. For culture cell assay, baicalein was added in culture medium containing 0.1% DMSO. For mouse assay, baicalein was intraperitoneally injected in mice containing 10% DMSO and 90% propylene glycol (0.8 mg/100 μL/mouse).

2.2. Reagents and Antibodies. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), ribonuclease A (RNase A), propylene glycol, MG-132, and crystal violet were purchased from Sigma (St. Louis, MO, USA). Anti-phospho-AKT(thr308), anti-phospho-AKT(ser473), and anti-cyclin D1 antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA). Anti-α-tubulin, anti-β-actin, and anti-phospho-GSK-3β(ser9) antibodies were purchased from GeneTex (Taichung, Taiwan). Anti-cyclin B1 was purchased from Epitomics (Burlingame, CA, USA). Anti-Bub3 was purchased from BD Biosciences (San Jose, CA, USA). Anti-p65NF-κB, anti-phospho-ERK(thr202/tyr204), and anti-phospho-p38(thr180/tyr182) were purchased from Cell Signaling Technology (Danvers,

MA, USA). The Millicell Hanging Cell Culture Inserts of Transwell system was purchased from Millipore (Billerica, MA, USA). Peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

2.3. Cell Viability Assay. Cell number was determined by colorimetric MTT assay. 5637 cells were cultured in 24-well plates at a density of 5×10^4 cells/well. After 24 h, cells were incubated with various concentrations of baicalein or 0.1% DMSO for another 24~72 h. Then MTT was added into medium for 2 h, the medium was discarded, and DMSO was added to dissolve the formazan product. Each well was measured by light absorbance at 490 nm. The result was expressed as a percentage, relative to 0.1% DMSO-treated control group.

2.4. Cell Cycle Analysis. Around 2×10^6 5637 cells were seeded in 100 mm dishes. After 24 h incubation for attachment, baicalein or DMSO was added. After baicalein treatment for 24 h and 48 h, cells were trypsinised, centrifuged, and fixed with ice-cold 75% ethanol overnight at 4°C. After removing the ethanol, cells were stained with a DNA staining solution (containing 1 mg/mL PI and 10 mg/mL RNase A dissolved in PBS) for 30 min at room temperature. The DNA content of the stained cells was measured using a FACScan flow cytometer. The cell doublets were removed by gating the left area of FL2-W/FL2-A plot for analysis. Cell cycle data from flow cytometry was analysed using ModFit LT software.

2.5. Cell Migration Assay. 5637 cells were seeded in 6-well plates. After cells had reached confluence, a wound was made by a 200 μL plastic tip in each well. The wells were then washed twice with PBS to remove cell debris and then incubated with culture medium with DMSO (control) or baicalein. After 24 h incubation, each well was photographed by a phase contrast microscopy. The empty area was calculated by computer, and the cell mobility was calculated by (scratch area − empty area of baicalein treatment) × 100%/(scratch area − empty area of control). Measurements were performed in triplicate and presented as mean ± SE from three independent experiments.

2.6. Cell Invasion Assay. The invasion assay was analyzed using a Matrigel (BD Biosciences)-coated Transwell system (Millipore). The upper chamber of the transwell was coated with 25 μg Matrigel. 5637 cells (1×10^5) in serum-free RPMI-1640 media were seeded onto Matrigel-coated Transwell. The upper and lower chamber media were added baicalein or 0.1% DMSO. In the lower chambers, 10% FBS was added as a chemoattractant. After a 24 h incubation time, the cells that remained on the upper surface of the filter membrane were removed, and the cells on the opposite surface of the filter membrane were stained with 4% paraformaldehyde for 30 s and photographed under microscopy at 200x magnification. The number of migrated cells was counted in five randomly chosen microscope fields.
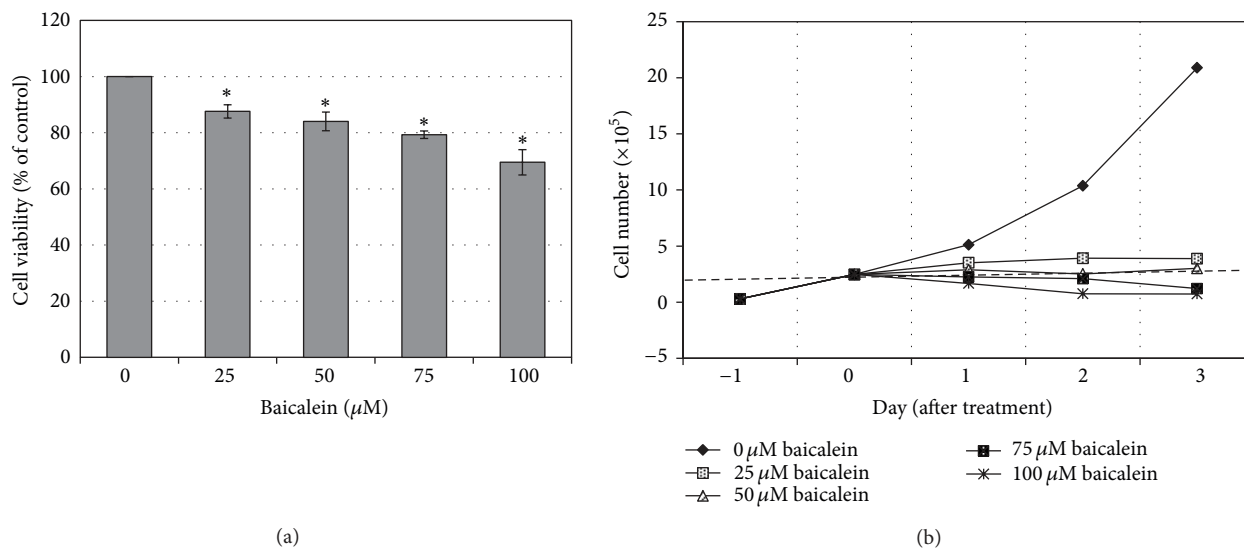
(a)

(b)

FIGURE 1: Effect of baicalein on cell growth. (a) Cytotoxicity of baicalein in 5637 bladder cancer cells. 5637 cells were initially seeded at 1×10^5 cells per well in 24-well plates and then treated with various concentrations of baicalein or vehicle (0.1% DMSO) for 24 h. The cell viability was measured by MTT assay. Measurement is performed from three independent experiments ($^*P < 0.05$ compared with vehicle). (b) Baicalein dose-dependently inhibits cell growth of 5637 cells. Cells were initially seeded at 1×10^5 cells (day-1) per well in 24-well plates and then treated with various concentrations of baicalein or vehicle (0.1% DMSO) for 24~72 h. The cell number was counted by trypan blue dye exclusion assay. The dotted line indicates the cell number on day 0.

2.7. RT-PCR. Total RNA was isolated from cells. Reverse transcription (RT) was performed on 2μg of total RNA by 1.5μM random hexamer and RevertAid reverse transcriptase (Fermentas); then 1/20 volume of reaction mixture was used for PCR with MMP-2 specific primers (5′CTT-CCAAGTCTGGAGCGATGT3′, 5′TACCGTCAAAGG-GGTATCCAT3′), MMP-9 specific primers (5′AAGATG-CTGCTGTTCAGCGGG3′, 5′GTCCTCTGGGCACTG-CAGGAT3′), and GAPDH specific primers (5′CGG-ATTTGGTCGTATTGG3′, 5′AGATGGTGATGGGAT-TTC3′). The PCR products were analyzed by 1% agarose gel.

2.8. Gelatin-Zymography Assay. The enzymatic activities of MMP-2 and MMP-9 were determined by gelatin-zymography. 3×10^6 cells were seeded in 10 cm dish for 24 h and then maintained in serum-free medium with various concentrations of baicalein. The conditioned medium was collected 24 h after drug treatment, concentrated by using an Amicon Ultracel YM-10 filter. Twenty micrograms of protein obtained from the concentrated medium was mixed with nonreducing sample buffer and subjected to electrophoresis (8% SDS-PAGE copolymerize with 0.1% gelatin as substrate). The gel was washed twice (15 min/time) with 2.5% Triton X-100 and incubated at 37°C for 16~20 h in 50 mM Tris-HCl (pH 7.8), 10 mM CaCl$_2$, and 0.01% NaN$_3$. The gel was stained with 0.15% Coomassie brilliant blue R-250 and destained in 50% methanol and 10% acetic acid until the gelatinolytic activities were detected as clear bands against a blue background.

2.9. Mouse Orthotopic Bladder Tumor Model. The female C57BL/6 mice aged five to six weeks were provided by the National Laboratory Animal Center (Taipei, Taiwan) and maintained at our animal care facility for one week prior to use. The implantation of murine bladder cancer cells MB49 into C57BL/6 mice was carried out similarly as previous report [17, 18]. *After MB49 inoculation (day 1),* mice were randomly assigned to two groups (10 mice/group). One group was intraperitoneally treated with vehicle (10% DMSO and 90% propylene glycol), and the other group received 0.8 mg/mouse baicalein intraperitoneally for 9 times. At the 21th day, the mice were sacrificed and the bladder volumes were measured before formalin fixation. After cutting into 4μm sections, the slides of each mouse were confirmed under a microscope in histology by hematoxylin and eosin staining. The experiment was approved by the Institutional Animal Care and Use Committee of National Chiayi University.

2.10. Statistical Analysis. The values shown are mean ± SEM. Data are statistically evaluated by one-way ANOVA of SigmaPlot 11.0 and shown significantly different in $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3. Results

3.1. Cytotoxicity and Proliferation Inhibition of Baicalein in 5637 Bladder Cancer Cells. Cytotoxicity of baicalein was analyzed by MTT assay. The result shows that baicalein dose-dependently inhibits cell viability after 24 h treatment (Figure 1(a)). Below 50μM, baicalein did not induce cell death because there were no floating cells after treatment. When the concentration reached 100μM, baicalein causes 33% cell number down with dead floating cells in the culture

Figure 2: Effect of baicalein on cell cycle phase distribution in 5637 cells. (a) Baicalein induces cell cycle arrest. Cells were treated with vehicle or baicalein for 24 and 48 h, then were collected for cell cycle analysis ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle). (b) Effect of baicalein on cyclin B1/D1 expression. Left, cells were treated with baicalein for 24 h. Right, cells were treated with 100 μM baicalein for 2, 6, 12, and 24 h.

(a)

(b)

(c)
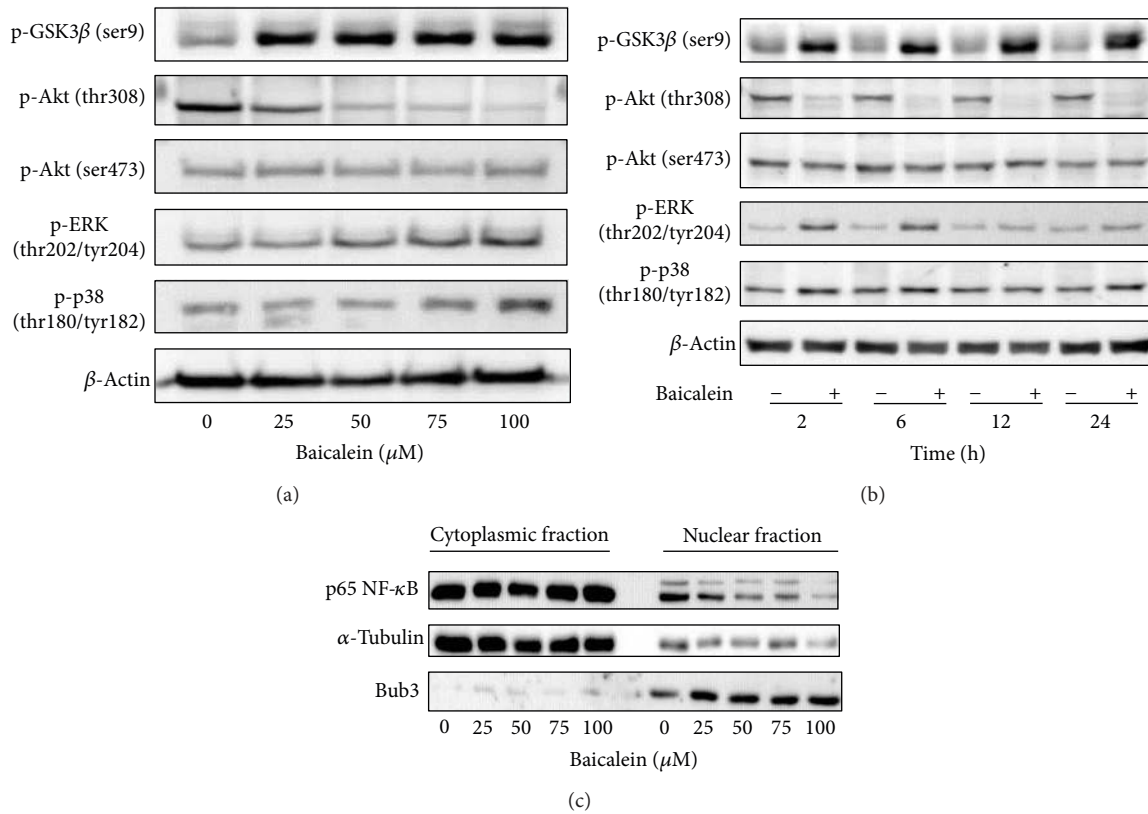
Figure 3: Influence of baicalein on the signal pathways. (a) Effect of baicalein on the phosphorylation of GSK3β, AKT, ERK, and p38. 5637 cells were treated with 0.1% DMSO or baicalein for 24 h. (b) Time-course of baicalein-changed signal protein phosphorylation. 5637 cells treated with 100 μM baicalein or 0.1% DMSO for 2, 6, 12, and 24 h. The total cell lysates were extracted for western blot analysis. β-Actin was used as a loading control. (c) Effect of baicalein on the nuclear p65NF-κB expression. 5637 cells were treated with 0.1% DMSO or baicalein for 24 h. The cytoplasmic and nuclear extracts were prepared for Western blot analysis. α-Tubulin and Bub3 are the loading control of cytoplasmic and nuclear fraction, respectively.

3.6. Baicalein Slightly Inhibits Tumor Growth with Some Hepatotoxicity in a Mouse Orthotopic Bladder Tumor Model In Vivo. Based on the antigrowth and antimetastasis activity of baicalein in cell assay, the in vivo antitumor assay was analyzed. After bladder cell implantation on day 1, baicalein treatment started on day 8. The treatment did not show toxicity in appearance and body weight (Figure 6(a)). Baicalein did not significantly reduce bladder size, but the mean bladder volume was still reduced in baicalein-treated mice (from 49.5 mm^3 to 35.9 mm^3 in Figure 6(b)). The blood biochemical analysis shows no significant change in serum BUN and creatinine between control and baicalein treatment groups, a little increase in GPT value but without statistical significance, and a significant increase in serum GOT (Table 1). It suggests that baicalein treatment causes some hepatic toxicity in mice.

4. Discussion

This study provides some new information about baicalein used in the anticancer therapy. In cell study, baicalein decreases cyclin B1 protein expression through inhibiting de novo protein synthesis and inhibits cyclin D1 by inhibiting protein synthesis and promoting proteasomal degradation.

Table 1: Effect of vehicle and baicalein on the plasma biochemical parameters of mice at termination of treatment. Values are mean ± SE.

	Vehicle	Baicalein
Blood urea nitrogen (BUN) (mg/dL)	22.8 ± 2.7	22.7 ± 1.8
Creatinine (mg/dL)	0.39 ± 0.01	0.39 ± 0.01
Glutamate oxaloacetate transaminase (GOT) (U/L)	66.0 ± 6.5	108.1 ± 16.6*
Glutamate pyruvate transaminase (GPT) (U/L)	27.2 ± 1.7	44.4 ± 3.6

*$P < 0.05$ compared with vehicle.

Baicalein-inhibited cyclin B1 is partially mediated by ERK activation. Among the signal transduction molecules AKT, GSK3β, ERK, p38, and p65NF-κB, p65NF-κB inhibition plays the most important role in baicalein-reduced cell viability. In mouse orthotopic bladder tumor model, baicalein has a little inhibition effect on orthotopic bladder tumor growth but with some hepatic toxicity.

Baicalein produces different cytotoxicity in different cell lines. For example, it causes cell cycle arrest at G1 phase
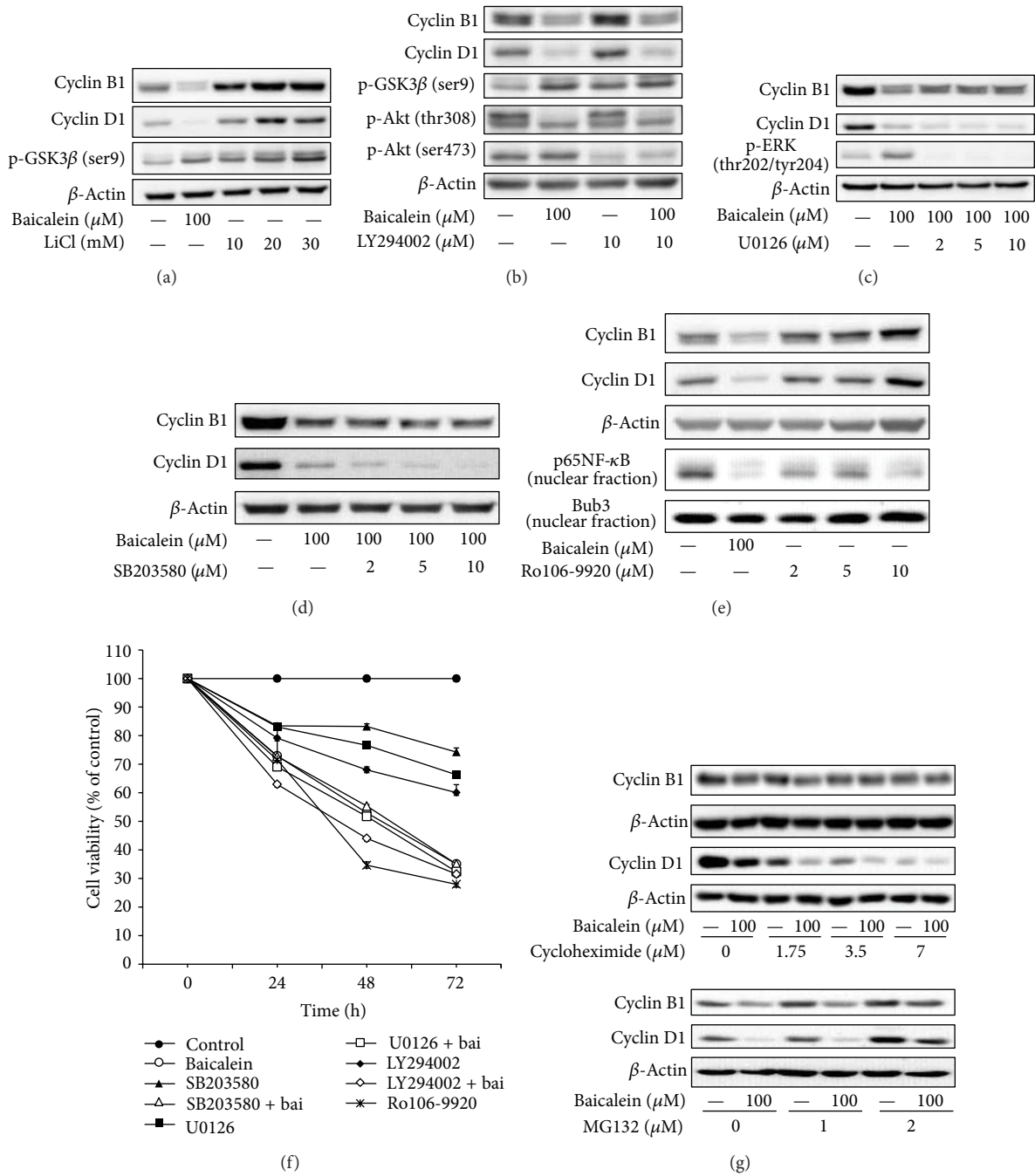
Figure 4: Effect of various inhibitors on baicalein-reduced cyclin B1/D1 expression. (a–e) Effect of LiCl (a), LY294002 (b), U0126 (c), SB203580 (d), and Ro106-9920 (e) on baicalein-reduced cyclin B1/D1 expression. (f) Effect of various inhibitors on baicalein-inhibited cell viability. All above inhibitors were pretreated for 1 h and baicalein treatment for 24~72 h. The concentration of each chemicals: baicalein is 100 μM and others are 10 μM. (g) Effect of cycloheximide or MG132 on baicalein-reduced cyclin B1/D1 expression. Cycloheximide was pretreated for 30 min and baicalein treatment for 1 h in cyclin D1 detection and baicalein treatment for 6 h in cyclin B1 detection. MG132 was pretreated for 1 h and baicalein treatment for 6 h. The extracted cell lysates or nuclear proteins were analyzed by western blot.

in breast cancer [21] and oral squamous cell carcinoma [22], at S phase in lung nonsmall carcinoma cell [23] and at G2/M phase in BFTC905 bladder cancer cells [9]. The differences may be caused by different doses and different cells used. In 5637 bladder cancer cells, baicalein arrests cells at S phase under 75 μM and at G1 phase at 100 μM without

apoptotic cells (Figure 2(a)). In pancreatic carcinoma PaCa cells [11], bladder cancer BFTC905 cells [9], and colorectal carcinoma HCT116 cells [10], baicalein induces cell apoptotic death at the dose between 5 to 60 μM. Baicalein also has a wide range on cytotoxicity of different cell lines, the IC$_{50}$ is under 20 μM in gastric cancer cells AGS and MKN-28 [24],

(a)

(b)

(c)

(d)
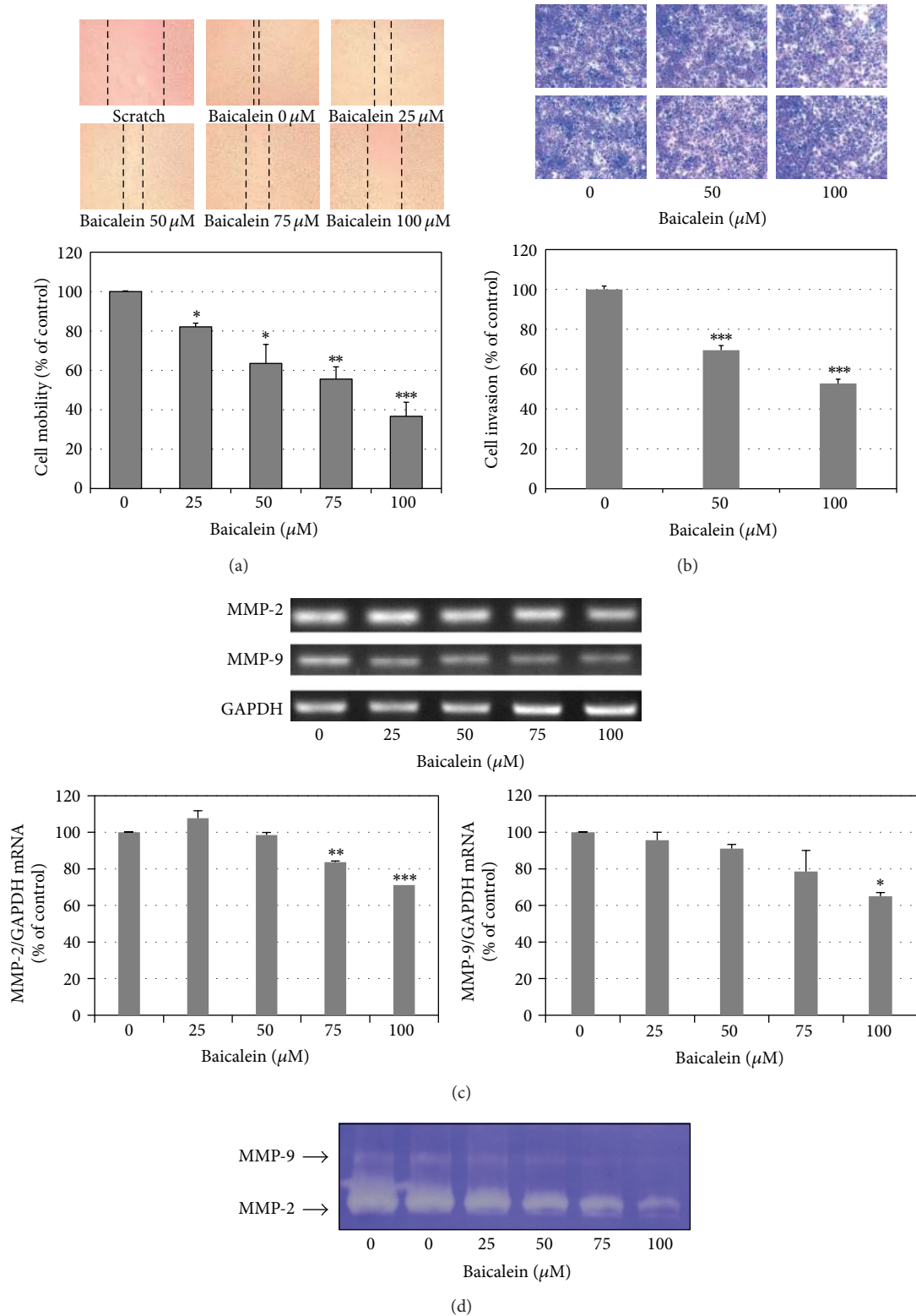
FIGURE 5: Anti-migration and anti-invasion activities of baicalein in 5637 cells. (a) Baicalein inhibits 5637 cell migration. Bottom chart is the percentage of migrated cells as control is 100%. (b) Baicalein inhibits 5637 cell invasion. Bottom chart is the percentage of invaded cells as control is 100%. (c) Effect of baicalein on the mRNA expression of MMP-2 and MMP-9. Bottom charts are the quantitative results from three independent experiments. (d) Effect of baicalein on the activities of MMP-2 and MMP-9. The conditioned medium was collected 24 h after drug treatment. Twenty micrograms of protein obtained from the concentrated medium was analyzed by gelatin-zymography assay. ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle).

(a)

(b)
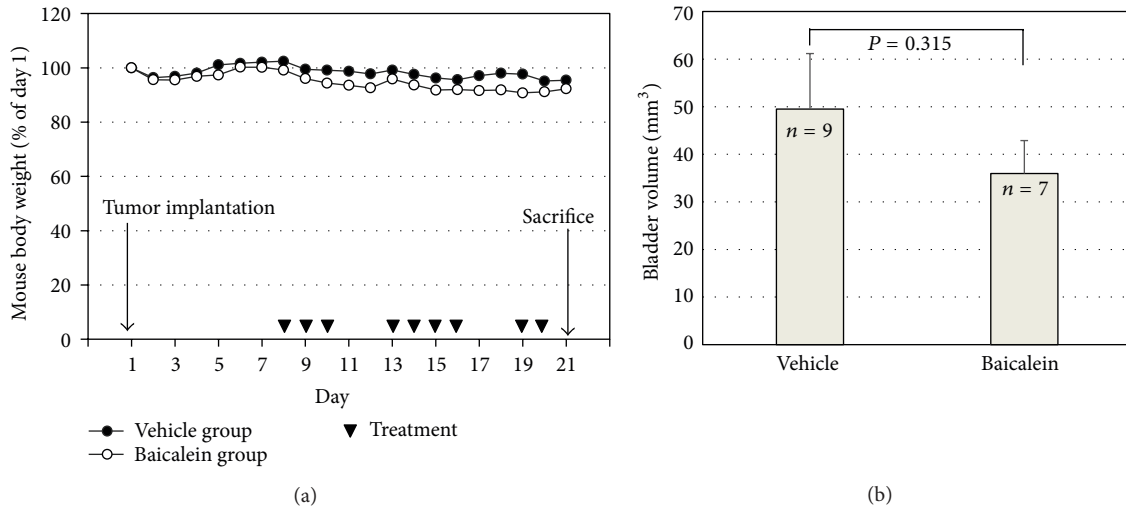
FIGURE 6: The antitumor effect of baicalein *in vivo*. (a) Mouse body weight and drug schedule in the mouse orthotopic bladder tumor model. After MB49 cell implantation, baicalein or vehicle was applied by intraperitoneal injection. Mouse body weight was recorded every day. At the 21th day, the mice were sacrificed. (b) Effect of baicalein in reducing bladder tumor size. The bladder volume of each mouse was measured. There are 9 mice survived at the 21th day in vehicle group and 7 in baicalein group.
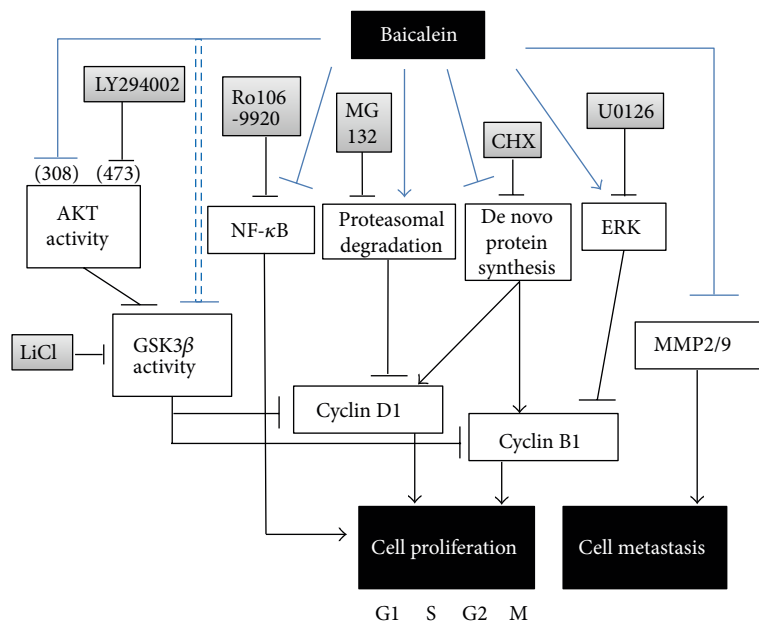
FIGURE 7: The pathway scheme of baicalein on the cell proliferation inhibition and cell metastasis inhibition in human bladder cancer cell 5637. The anti-proliferation and anti-metastasis activities of baicalein may contribute the anti-bladder-tumor effect *in vivo*.

prostate carcinoma LNCaP [25], and JCA-1 [26], between 20 to 50 μM in leukemia HL-60 [27], bladder cancer BFTC905 [9], hepatic cancer Hep G2 [28], and myeloma cell U266 [29], and more than 100 μM in 5637 bladder cancer (Figure 1(a)), oral squamous carcinoma HSC-3 [22], leukemia THP-1, and osteogenic cancer cell HOS [30]. Although the detail mechanisms about the wide-range cytotoxicity are still unclear, this property may provide a specific and lower hazard anticancer effect for the higher sensitive tumors.

PI3K, the upstream signal of AKT, has been reported to be inhibited by baicalein [31]. In our study, baicalein inhibits pAKT(thr308) phosphorylation and has no influence on pAKT(ser473) (Figure 2(b)). The pAKT inhibition phenomenon also has been reported in prostate cancer cell DU145 [32] and oral squamous carcinoma HSC-3 [22]. On the other hand, the pAKT(ser473) activation has been reported in bladder cancer BFTC905 [9, 10]. Because the pAKT(thr308) phosphorylation site is the direct target site for PI3K-PDK1 [33], it will be downregulated after PI3K inhibition by baicalein (Figure 4(b)). The ser473 site of AKT is phosphorylated by rictor-mTOR [34], not PI3K; therefore, it may be the reason for the no effect of baicalein

on the phosphorylation of pAKT(ser473). Even though the pAKT(thr308) is decreased by baicalein, pGSK3β(ser9), one AKT downstream [35], is still phosphorylated by baicalein (Figure 3(b)). Because the phosphorylation of pGSK3β(ser9) is achieved by numerous kinases, not only AKT [35], baicalein-induced pGSK3β(ser9) may be caused by the influence of other kinase(s).

NF-κB, an important inflammatory transcription factor, is inhibited by baicalein in 5637 cells (Figure 3(c)). Baicalein-inhibited p65NF-κB activation has also been reported in human mast cells [36], mouse macrophages [37, 38], human myeloma cells [29], and brain microglia [39, 40]. According to the important role of p65NF-κB in tumor progression and metastasis [41, 42], the inhibition of baicalein on nuclear NF-κB is a critical function in its anti-inflammation and anticancer application. In human hepatoma cells, baicalein shows anti-migration property with NF-κB inhibition [12]. There is one report indicates that GSK3β inhibition results in inhibiting NF-κB activity [43]; therefore, the mechanism of baicalein-inhibited p65NF-κB activity may be partially mediated by baicalein-inhibited GSK3β. In Figure 4(f), among the 4 signal inhibitors, the cell viability decreases at most by NF-κB inhibitor Ro106-9920, which indicates that NF-κB is a critical factor for proliferation of 5637 cells.

Baicalein inhibits the protein expression of cyclin B1 [9, 23, 44] and cyclin D1 [22, 32, 44], has also been reported by some studies, but the mechanism is still unclear. In this study, we first suggest that baicalein decreases cyclin B1 expression through inhibiting *de novo* protein synthesis but not promoting proteasomal degradation and decreases cyclin D1 by both ways (Figure 4(g)). On the other hand, cyclin B1 reduction is partially mediated by ERK activation (Figure 4(c)). Luteolin, a natural flavonoid with structure similar to baicalein, decreases cyclin D1 expression by increasing proteasomal degradation [45]. Though the structures are similar between baicalein and luteolin, the mechanisms for cyclin D1 reduction are different. Luteolin enhances proteasomal degradation via decreasing GSK3β(ser9) phosphorylation, but baicalein increases GSK3β(ser9) phosphorylation (Figures 3(a) and 3(b)). Therefore, there is (are) other pathway(s) for inducing cyclin D1 degradation by baicalein.

In addition to anti-proliferation, baicalein also inhibits cancer cell metastasis. Either in scratch assay (Figure 5(a)) or in Matrigel-coated transwell assay (Figure 5(b)), they point out the anti-migration and anti-invasion property of baicalein. In this study, we confirm this inhibition, like others [12, 13], mediated by inhibiting MMP-2/9 activities (Figure 5(d)). The correlated signal pathways need to be further investigated. In the orthotopic bladder tumor model, baicalein shows a little effect on inhibiting bladder tumor growth (Figure 6). One report indicates that baicalein significantly reduces tumor volume in a nude mice model [12]. Comparing these two animal models, we use higher dose and lower frequency of baicalein, which may result in the lower efficiency. But it still notices that baicalein induces hepatic toxicity with GOT value increase. In order to avoid hepatic toxicity, it is better to use baicalein locally, for example, by intravesical application for bladder tumor therapy.

5. Conclusions

In this study, baicalein decreases cyclin D1 protein expression through inhibiting *de novo* protein synthesis and promoting proteasomal degradation and decreases cyclin B1 by inhibiting *de novo* protein synthesis. Baicalein-inhibited cyclin B1 expression is slightly mediated by ERK activation. The mechanism of baicalein in anti-proliferation and anti-metastasis is concluded in Figure 7. Among the signal transduction molecules AKT, GSK3β, ERK, p38, and p65NF-κB, p65NF-κB inhibition plays the most important role in baicalein-reduced cell viability. In mouse orthotopic bladder tumor model, baicalein has a little effect on orthotopic bladder tumor growth inhibition but with some hepatic toxicity.

Abbreviations

FBS: Fetal bovine serum
MMP-2: Matrix metalloproteinase-2
MMP-9: Matrix metalloproteinase-9
MTT: 3-(4,5-dimethylthiazol-2-yl)2,5-
             diphenyltetrazolium bromide
NF-κB: Nuclear factor-kappaB
PI3K: Phosphatidylinositol 3-kinase.

Conflict of Interests

The authors declare that there is no conflict of interests.

Authors' Contribution

J.-Y. Wu and K.-W. Tsai contributed equally to this work.

Acknowledgments

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