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Baicalein induces a dual growth arrest by modulating multiple cell cycle regulatory molecules

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Abstract

Baicalein, a flavonoid present in the root of *Scutellaria baicalensis* Georgi, has been reported to inhibit cell proliferation in several types of cells. In this study, the effect of baicalein on cell growth and the mechanism of growth modulation were examined in primary cultured rat heart endothelial cells. Here, we report that treatment with 100-μM baicalein caused an almost complete inhibition of cell proliferation after 5 days of incubation. Baicalein mediated G1 and G2 growth arrest accompanied by the down-regulation of cyclin D2, cyclin A, cyclin-dependent kinase 1 (Cdk1) and cyclin-dependent kinase 2 (Cdk2), and up-regulation of $p15^{Ink4B}$, $p21^{CIP1/Waf1}$, p53 and cyclin E. Evaluation of the kinase activity of cyclin–Cdk complexes showed that baicalein decreased Cdk1, Cdk2, cyclin D2 and cyclin A expression in endothelial cells, leading to markedly reduced Cdk/cyclin-associated kinase activities. These results suggest that baicalein inhibits the proliferation of rat heart endothelial cells via G1 and G2 arrest in association with the down-regulation of the expression and function of Cdk1, Cdk2, cyclin D2 and cyclin A proteins, and up-regulation of cyclin E, $p15^{Ink4B}$, p53 and $p21^{CIP1/Waf1}$.

Keywords: Baicalein; Cyclin; Cyclin-dependent kinase; $p15^{Ink4B}$; p53; $p21^{CIP1/Waf1}$

1. Introduction

The root of *Scutellaria baicalensis* Georgi (Chinese name: Huangqin), a conventional Chinese herbal medicine, contains numerous flavones. Baicalein (5,6,7-trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one), a major flavonoid from Huangqin, has attracted considerable attention because it has a variety of interesting activities such as antibacterial (Kubo et al., 1981), antiviral (Austin et al., 1992), lipoxygenase inhibitory (Marshall et al., 1993; Gao et al., 1996), hypotensive (Tang and Zhou, 1958), and anticancer effects (So et al., 1997). Baicalein has been shown to effectively inhibit the growth of several types of cells (Qain et al., 1989; Huang et al., 1994; Inoue and Jackson, 1999) and to induce cell death in human hepatocellular carcinoma cell lines (Matsuzaki et al., 1996). Increased cell proliferation is one of the key abnormalities in the development of many diseases such as rheumatoid arthritis, inflammation, atherosclerosis and cancers. Recent efforts have focused on the isolation of naturally occurring clinical therapeutic agents for cancers. The most intriguing property of baicalein with respect to cancer therapy is its ability to inhibit cell growth and induce cell death. The antiproliferative activity of baicalein has been well documented (Qain et al., 1989; Huang et al., 1994; Inoue and Jackson, 1999); however, the mechanism by which it specifically inhibits cell growth in vitro and/or in vivo remains largely uncharacterized.

The modulated expression of the cell cycle regulatory genes is one of the important mechanisms of cell growth inhibition (Nigg, 1995). The cell cycle is regulated by the coordinated action of cyclin-dependent kinases (Cdks) in association with their specific regulatory cyclin proteins (Nigg, 1995). Thus, functional activation of Cdk–cyclin kinase activities is required for cell cycle progression. The kinase activity of these Cdk–cyclin complexes is inhibited by Cdk inhibitors, including $p15^{Ink4B}$, $p16^{Ink4A}$, $p21^{CIP1/Waf1}$, and $p27^{Kip1}$ (Nigg, 1995). Our preliminary data showed that baicalein strongly inhibited the growth of primary cultures of rat heart endothelial cells.

The proliferation of endothelial cells leads to angiogenesis, which facilitates tumor initiation and promotion

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(Bouck et al., 1996). Angiogenesis inhibitors represent a promising new class of therapeutic agents against cancer (Bouck et al., 1996). It is of interest to define whether the antiproliferative effect of baicalein is caused by an effect on endothelial cell cycle progression. Thus, we attempted to explore the mechanism(s) by which baicalein mediates growth inhibition, through a detailed analysis of the effects of baicalein on cell cycle regulatory proteins. The results demonstrated that the baicalein-mediated S phase decrease was concomitant with a G1 and G2 phase increase in rat heart endothelial cells. A number of cell cycle regulatory proteins, such as Cdk1, Cdk2, cyclin D2, cyclin A, cyclin E, p53, $p15^{Ink4B}$ and $p21^{CIP1/Waf1}$ were identified as downstream targets in the growth-inhibition activity of baicalein.

2. Materials and methods

2.1. Materials

Culture medium and fetal bovine serum were from Gibco BRL. Anti-Von Willebrand factor antibody and fibronectin were from Boehringer-Mannheim. Baicalein, propidium iodide and endothelial cell growth supplement were obtained from Sigma. One hundred millimolar baicalein was dissolved in dimethyl sulfoxide (DMSO) and stored in a liquid nitrogen tank. Fresh baicalein was included in replacement medium if baicalein treatment duration was longer than 48 h. Anti-Cdk1, Cdk2, Cdk3, Cdk4, anti-cyclin B, D1, D3, anti-$p21^{CIP1/Waf1}$, and anti-$p27^{Kip1}$ were obtained from Transduction Laboratory. Anti-cyclin A, anti-cyclin D2, anti-cyclin E, anti-Cdk6, anti-$p15^{Ink4B}$, and anti-$p16^{Ink4A}$ were obtained from Santa Cruz. Anti-pRb and anti-p53 were purchased from Pharmingen.

2.2. Rat heart endothelial cell culture preparation

Rat heart endothelial cells were isolated as described previously (Richards et al., 1986). Briefly, rat hearts were removed from 4-day-old donors, then washed with Hanks balanced salt solution (HBSS) and finely minced with dissecting scissors. The tissue was subjected to four successive trypsinization (10 ml 0.125% trypsin in calcium- and magnesium-free HBSS) steps under stirring in a 50-ml flask. After each trypsinization, free cells were removed and placed in 2 ml of endothelial cell culture medium containing Dulbecco's modified essential medium (DMEM) and 15% fetal bovine serum. The cells were collected by centrifugation at $1000 \times g$, then resuspended in endothelial cell culture medium, and allowed to adhere to the fibronectin-coated plates for 90 min. The culture plates were washed with HBSS to remove nonadherent cells (mostly myocardiocytes). Culture medium (supplemented with 50 μg of endothelial cell growth supplement per milliliter, 15% fetal bovine serum) was added to the remaining adherent cells (endothelial cells).

2.3. Morphological investigation

Rat heart endothelial cells were untreated or treated with 10 or 100 μM baicalein for 3 days. After treatment, cells were stained with Giemsa solution, and morphology was investigated and photographed using an Olympus IX70 microscope.

2.4. Cell proliferation and DNA synthesis assay

Rat heart endothelial cells were seeded onto 12-well plates at 1×10^4 cells/well. After 24 h, cells were treated with various concentrations of baicalein for the indicated time periods. Cell number was determined by Trypan blue dye exclusion, using a hemocytometer. Cellular DNA synthesis was monitored in cultures treated with vehicle alone (0.1% DMSO) and in cultures treated with baicalein, at the indicated experimental time points. DNA synthesis assay was performed as previously described (Hsu et al., 2000). [^{3}H]Thymidine incorporation into DNA was measured with a liquid scintillation counter.

2.5. Cell cycle analysis

Cells were treated without or with baicalein for the indicated time periods, and cell cycle distribution was analyzed using flow cytometry. Briefly, 2×10^6 cells were trypsinized, washed with phosphate-buffered saline (PBS), and fixed in 80% ethanol. They were then washed with PBS, incubated with 100 μg/ml RNase at 37 °C for 30 min, stained with propidium iodide (50 μg/ml), and analyzed on a FACScan flow cytometer. The percentage of cells in different phases of the cell cycle was analyzed using Cell-FIT software (Becton Dickinson Instruments).

2.6. Protein preparation, immunoblotting and immunoprecipitation

Cells were cultured without or with 10 or 100 μM baicalein for the indicated time points. After treatment, cell extracts were prepared as described previously (Hsu et al., 2000); protein concentration was determined using the Bradford method. Equal amounts of sample lysate were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with specific primary antibodies, including anti-rat Cdks, cyclins, $p15^{Ink4B}$, $p16^{Ink4A}$, $p21^{CIP1/Waf1}$, $p27^{Kip1}$, p53, and pRb antibodies. Determinations were made using enhanced chemiluminescence kits (Amersham, ECL Kits) (Hsu et al., 2000). To prepare proteins for immunoprecipitation, cells were lysed in hypotonic buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 10% glycerol, 1 mM dithiothreitol, 0.1% NP-40, 10 mM β-glycerophosphate, 1 mM NaF, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin). Protein concentration was

determined and equal amounts of precleared cell lysates were mixed with protein A-Sepharose beads (Pharmacia) and mouse anti-rat Cdk-or cyclin-specific antibodies. After 4 h, samples were centrifuged and washed extensively with hypotonic buffer. Immunoprecipitated complexes were used for kinase assay.

2.7. Kinase assay

Immunocomplexes were assayed for pRb or histone H1 kinase activity by washing twice with kinase buffer, followed by incubation in 30 μl of kinase buffer containing 20 mM Tris–HCl, pH 7.5, 5 mM EGTA, 20 mM $MgCl_2$, 0.5 mM dithiothreitol, 1 μg histone H1 (or 1 μg pRb substrate peptide), 5 μCi [γ^{32}P]ATP (6000 Ci/mmol; DuPont NEN), and 1 μM ATP at 37 °C for 15 min. The reaction was terminated by the addition of 10 μl of 4 × Laemmli sample buffer, followed by boiling for 10 min. Proteins were subjected to SDS-PAGE, after transfer to nitrocellulose paper. The incorporation of ^{32}P was visualized by autoradiography and quantitated with a PhosphoImage (Molecular Dynamics).

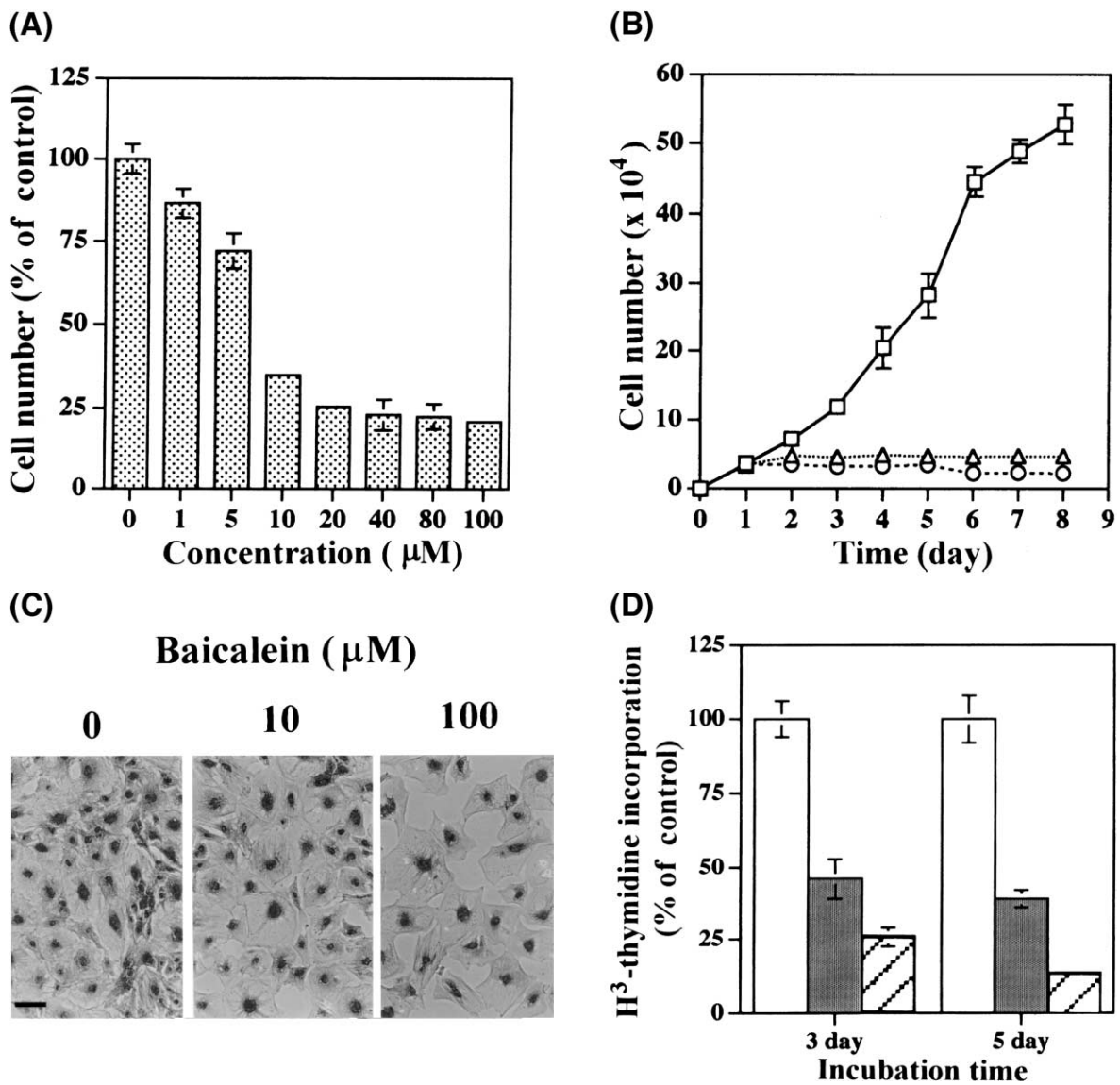

Fig. 1. Effect of baicalein on rat heart endothelial cells. (A) Concentration-dependent cell growth inhibition. Endothelial cells were cultured without or with various concentrations of baicalein (1, 5, 10, 20, 40, 80, 100 μM) for 5 days. After treatment, cell number was measured with the Trypan blue dye exclusion method. (B) Time-dependent cell growth inhibition. Cells were cultured without or with baicalein (10 and 100 μM), and cell number was determined daily. (C) Changes in cell morphology. Cells were treated with 0-, 10-, and 100-μM baicalein for 3 days. After incubation, cells were stained with Giemsa solution. The morphology was examined using an Olympus IX70 microscope. All photographs were taken at the same magnification of ×200. Scale bar, 25 μM. (D) Suppression of DNA synthesis by baicalein. Endothelial cells were plated onto 12-well plates at a density of 2×10^4 cells/well. After 24 h, cells were treated with 0 (white column), 10 (gray column) or 100 μM (striped column) baicalein for 3 or 5 days. [^{3}H]Thymidine incorporation into DNA was determined and expressed as percentage of control (0.1% DMSO treatment).

2.8. Analysis of data

All data are presented in this report as means ± S.D. of 12 replicates from four separate experiments. Statistical differences were evaluated using the Student's *t*-test and considered significant at the $P < 0.01$ or $P < 0.05$ level. All the figures shown in this article were obtained from at least four independent experiments with a similar pattern.

3. Results

3.1. Baicalein-mediated growth inhibition and cell cycle changes in endothelial cells

Rat heart endothelial cells were treated with various concentrations of baicalein (0, 1, 5, 10, 20, 40, 80, 100 μM) for 5 days, and then cell number was determined with the Trypan blue dye exclusion method. As depicted in Fig. 1A, baicalein drastically inhibited the proliferation of endothelial cells in a concentration-dependent manner. The number of cells in the 10- and 100-μM baicalein-treated cultures was reduced approximately 65% and 75%, respectively. The time course study of baicalein-mediated antiproliferation in rat heart endothelial cells revealed that prolonged exposure to baicalein was necessary to signal growth inhibition. The growth of endothelial cells was almost completely suppressed in the continuous presence of 100-μM baicalein, and the cell number was maintained approximately similar to that of seeded cells. It is also noteworthy that the control culture not treated with baicalein had a consistent increase in cell number (Fig. 1B). Moreover, the growth inhibition induced by baicalein did not seem to be due to general cytotoxicity, because very few floating dead cells were observed even at 100-μM baicalein. However, baicalein induced process-bearing endothelial cells to adopt a more large and flattened morphology in comparison with that of control cultures (regular, small oval shape and densely packed confluent cell layer) (Fig. 1C). To further investigate the effect on DNA synthesis of baicalein, endothelial cells were treated with baicalein (10 and 100 μM) or with 0.1% DMSO (vehicle control) for 3 and 5 days, and DNA synthesis was examined by [^{3}H]thymidine incorporation. Results showed that exposure to baicalein markedly suppressed DNA synthesis by endothelial cells (Fig. 1D). Baicalein at 10 and 100 μM caused 65% and 85% DNA synthesis inhibition after 5 days incubation, respectively.

3.2. Baicalein induced a dual growth arrest in endothelial cells

To more precisely determine the mechanism by which baicalein inhibits the proliferation of endothelial cells, flow cytometry analysis was performed on cells treated with 0-, 10-, and 100-μM baicalein for 3 and 5 days. As indicated in Fig. 2, baicalein treatment caused a time- and concentration-dependent decrease in the percentage of cells in S phase. The decrease in S phase cells after baicalein treatment was accompanied by an increase in G1 and G2/M populations, relative to control cultures. These results demonstrated that baicalein mediated G1 and G2/M phase arrest in endothelial cells.

3.3. Regulation of cell cycle regulatory proteins by baicalein

Cell cycle progression is tightly regulated through a complex network of positive and negative cell cycle regulatory molecules, such as Cdks, cyclins, Cdk inhibitors,

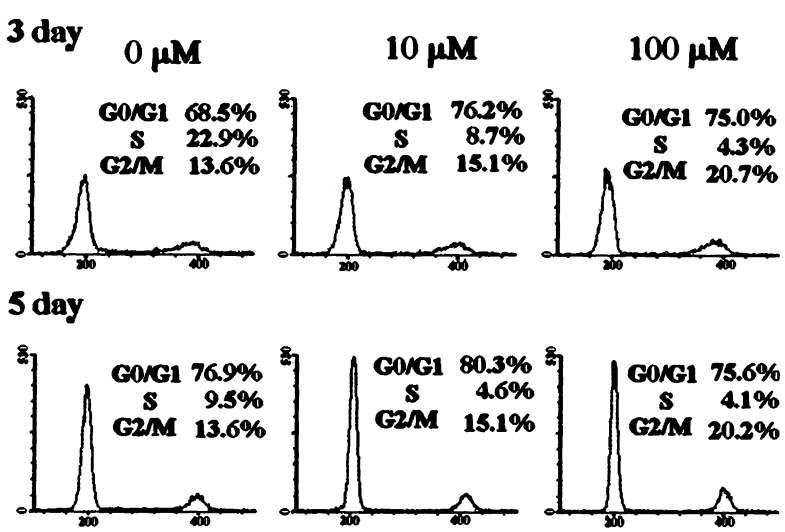

Fig. 2. Effect of baicalein on cell cycle distribution. Endothelial cells were treated with 0-, 10- or 100-μM baicalein for 3 or 5 days. After treatment, cells were harvested and stained with propidium iodide. Cell cycle distribution was analyzed using a FACScan flow cytometer. The distribution of cells in G1, S, G2/M was determined using Cell-FIT software. Percentage of cells in G1, S, G2/M is shown.

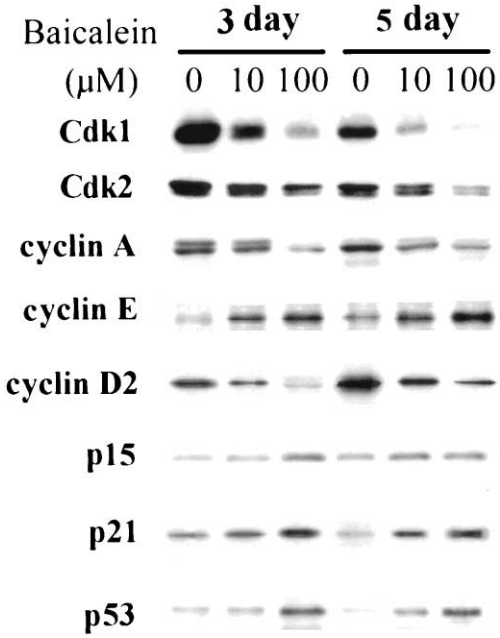

Fig. 3. Regulation of cell cycle regulatory proteins by baicalein. Endothelial cells were treated with 0-, 10-, or 100-μM baicalein. After 3 and 5 days of incubation, protein lysates were prepared. Western blotting analysis using antibodies directed against the indicated proteins.

p53 and pRb. To elucidate the specific cell cycle regulatory proteins responsible for the cell cycle block mediated by baicalein in endothelial cells, protein extracts were prepared from cells treated for 3 and 5 days. Western blot analysis was performed using antibodies specific for Cdks (Cdk1, 2, 3, 4, 6), cyclins (cyclin A, B, D1, D2, D3, E), CdkIs ($p15^{Ink4B}$, $p16^{Ink4A}$, $p21^{CIP1/Waf1}$, $p27^{Kip1}$), p53, and pRb proteins. We found that baicalein treatment caused a marked decrease in Cdk1, Cdk2, cyclin A, and cyclin D2 protein expression (Fig. 3), with no detectable changes in Cdk3, Cdk4, Cdk6, cyclin B, cyclin D1, cyclin D3, $p16^{Ink4A}$, $p27^{Kip1}$, and pRb protein levels (data not shown). In contrast, cyclin E, $p15^{Ink4B}$, $p21^{CIP1/Waf1}$, and p53 was significantly up-regulated in baicalein-treated endothelial cells. These results imply that the growth inhibition by baicalein in endothelial cells may target several components of the cell cycle regulatory apparatus.

3.4. Modulation of Cdk1, Cdk2, cyclin D2-, and cyclin A-associated kinase activities by baicalein

To elucidate whether the baicalein-induced down-regulation of Cdk1, Cdk2, cyclin D2, and cyclin A was associated with changes in the kinase activities of various cyclin–Cdk complexes, we performed in vitro kinase assays. When histone H1 or pRb (for cyclin D2-associated kinases) was used as a substrate in immunoprecipitation experiments performed with antibodies to Cdk1, Cdk2, cyclin A, and cyclin D2, lysates from cells treated with baicalein at 10 and 100 μM for 5 days showed a marked decrease in kinase activity (Fig. 4). At 100-μM baicalein treatment, the Cdk1, Cdk2, cyclin A-, cyclin D2-associated kinase activities were decreased approximately 4-, 4-, 4-, and 3-fold for 5-day treatments, respectively (Fig. 4). These results indicated that the baicalein-induced down-regulation of Cdk1, Cdk2, cyclin D2, and cyclin A was consistent with decrease in their kinase activities.

4. Discussion

Recent reports have been shown that baicalein inhibits cell growth in human hepatoma HuH-7 cells (Qain et al., 1989), suppresses the proliferation of vascular smooth muscle cells (Huang et al., 1994), and strongly inhibits the growth of cultured rat hepatic stellate cells (Inoue and

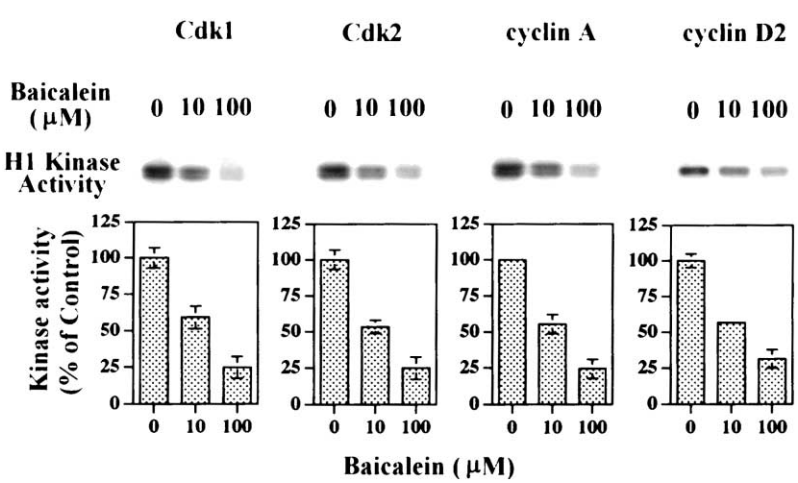

Fig. 4. Modulation of Cdk and cyclin-associated kinase activity by baicalein. Protein lysates from endothelial cells treated with 0-, 10-, and 100-μM baicalein for 5 days were subjected to immunoprecipitation with Cdk1-, Cdk2-, cyclin A-, or cyclin D2-specific antibodies and the resultant immunocomplexes were analyzed in kinase assays for activity against histone H1 (for Cdk1, Cdk2, and cyclin A) or pRb peptide (for cyclin D2). Quantitation was by PhosphoImage and fold activity was calculated relative to that found in vehicle-treated control cultures.

Jackson, 1999); however, the exact molecular mechanisms by which baicalein arrests cell growth are not known. In this study, we demonstrated that rat heart endothelial cell growth is also drastically inhibited by baicalein in the presence of serum and endothelial growth supplement. Moreover, the growth inhibition by baicalein did not seem to be due to general cytotoxicity, because floating dead cells and terminal transferase-mediated dUTP-fluorescensin nick end-labeling (TUNEL) positive cells were not detected during baicalein treatment (data not shown). Our studies revealed a unique mode of action of baicalein on endothelial cell proliferation and cell cycle progression. Endothelial growth supplement is a potent mitogen for vascular and capillary endothelial cells in vitro. Our results indicated that endothelial cell proliferation inhibition was independent of the nature of the growth stimuli used, suggesting that the growth inhibition induced by baicalein may be mediated through cell cycle events. Data from flow cytometry analysis also suggest that the cell growth inhibition induced by baicalein was mediated through the cell cycle. The percentage of endothelial cells in S-phase decreased concomitantly with an increase in cells in the G1 and G2/M phases upon baicalein treatment.

Cell cycle control is a highly regulated process that involves a complex cascade of events. Modulation of the expression and function of the cell cycle regulatory proteins (including Cdks, cyclins, CdkIs, p53, and pRb) provides an important mechanism for inhibition of growth (Nigg, 1995). The fact that baicalein permitted endothelial cells to arrest in G1 and G2/M phases suggests that modulation of several cell cycle regulatory proteins may occur after baicalein treatment. Here, we showed that, in response to baicalein, there is an up-regulation of cyclin E, p53, $p15^{Ink4B}$ and $p21^{CIP1/Waf1}$ proteins, and the down-regulation of the Cdk1, Cdk2, cyclin D2, and cyclin A proteins and their kinase activities correlated with a decrease in DNA synthesis and cell growth inhibition, paralleling the S-phase decrease. These observations suggest that the antiproliferative activity of baicalein in rat heart endothelial cells is related to a multifaceted attack on multiple target molecules that are critically involved in growth inhibition.

In mammalian cells, the key G1 phase Cdks are Cdk4 (and its functional counterpart, Cdk6) and Cdk2 (Sherr and Roberts, 1995). Cdk4/6 and Cdk2 are inactive in the absence of their cyclin partner(s), and they are activated by the binding of D type cyclins (D1, D2, D3 to Cdk4/6) or cyclin E (to Cdk2) (Sherr and Roberts, 1995). Over-expression of D-type cyclins can shorten the G1 interval (Sherr, 1995). Microinjection of antisense plasmids or antibodies to cyclin D into normal fibroblasts can prevent them from entering S phase but is without effect once cells approach the G1/S boundary (Sherr, 1995). D-type cyclins therefore execute their critical functions during mid-to-late G1 phase. Based on these data, we suggest that the baicalein-mediated decrease in cyclin D2 protein and its associated kinase activity may permit endothelial cells to arrest in G1 phase. Yin et al. (1999) reported that increased cyclin E protein expression and its associated kinase activity was accompanied by an inhibition of DNA synthesis. These results suggest that cyclin E–Cdk2 kinase can negatively regulate DNA replication. In addition, cyclin A is induced at or near the G1/S boundary; it binds to Cdk2 in S phase and is involved in S phase progression (Girard et al., 1991; Pagano et al., 1992). Microinjection of antisense cDNA or antibodies to cyclin A in G1 phase cells blocks subsequent DNA synthesis (Girard et al., 1991; Pagano et al., 1992). Here, we showed that the increase in cyclin E protein was accompanied by an inhibition of DNA synthesis in baicalein-treated endothelial cells, while Cdk2 and cyclin A protein and their associated kinase activities decreased to very low levels. It could be that up-regulation of cyclin E and down-regulation of cyclin A and Cdk2 interrupted DNA synthesis, leading to growth arrest in the G1/S boundary upon baicalein treatment.

In addition to cyclin binding, the activity of the cyclin/Cdk complex is affected by the action of specific cyclin-dependent kinase inhibitors (CdkIs). There are two CdkI families, CIP/KIP and INK. $P21^{CIP1/Waf1}$, one of the CIP/KIP family members, is a down-stream target of p53. It can tightly bind and inhibit the kinase activities of several cyclin–Cdk complexes, such as cyclin D–Cdk4/6, cyclin E–Cdk2 and cyclin A–Cdk2, inhibits their kinase activities, and arrests cell growth at the G1 and G1/S boundary (Sherr and Roberts, 1995). $P15^{Ink4B}$, an INK family member, which binds only to cyclin D–Cdk4/6, inhibits their kinase activities and also arrests cell growth in the G1 phase (Sherr and Roberts, 1995). Thus, another possible explanation of the altered cyclin–Cdk activity and arrested rat heart endothelial cell growth in the G1 phase after baicalein treatment is the up-regulation of $p15^{Ink4B}$, p53 and $p21^{CIP1/Waf1}$ protein expression.

It is noteworthy that baicalein treatment increased the G2/M population of endothelial cells, but did not increase the mitotic index (data not shown), suggesting that baicalein treatment caused an accumulation of cells in the G2 phase. Morphological observations revealed similar results. These data suggest that the G2/M accumulation induced by baicalein does not reflect M arrest, but rather results from G2 phase arrest. The progression of cells from G2 into mitosis is mainly controlled by the formation and activation of the Cdk1/cyclin B complex (Nigg, 1995). Previous reports demonstrated that suppression of Cdk1 and cyclin B levels and inhibition of Cdk1 kinase activity are involved in G2 delay after genotoxic stress (Villa et al., 1996; Halloran and Penton, 1998). Our findings are consistent with these results. Baicalein, which decreased the expression of Cdk1 protein and inhibited the Cdk1 kinase activity in rat heart endothelial cells, causes cell cycle arrest in G2 because the Cdk1 kinase activity necessary for the transition of cells into the mitotic phase is reduced. It is

known that increased expression of p53 and $p21^{CIP1/Waf1}$ can arrest cells in both G1 and G2 phases (Schmidt et al., 2000). In G2, $p21^{CIP1/Waf1}$ expression may indirectly block Cdk1/cyclin B kinase activity and prevent progression into the M phase (Sherr and Roberts, 1995). Therefore, the up-regulation of p53 and $p21^{CIP1/Waf1}$ may provide another explanation for the baicalein-mediated G2 arrest in endothelial cell cultures.

Taken together, these data suggest that the G1 and G2 arrest of rat heart endothelial cells following baicalein treatment is mainly due to the loss of the Cdk kinase components including cyclin D2, cyclin A, Cdk1, and Cdk2, and also result from the inhibitory action of $p15^{Ink4B}$ and $p21^{CIP1/Waf1}$ on the Cdk complexes. Changes in the control of vascular endothelial cell proliferation are an important factor in angiogenesis (Shibuya, 1995; Stromblad and Cheresh, 1996). Under physiological conditions, new blood vessels are formed during the body's repair processes such as wound healing and embryonic development (Shibuya, 1995; Stromblad and Cheresh, 1996). However, uncontrolled angiogenesis is pathological and is often associated with conditions such as rheumatoid arthritis, psoriasis, diabetic retinopathy, hemangiomas, inflammation, tumor growth (Shibuya, 1995; Bouck et al., 1996; Stromblad and Cheresh, 1996), and atherosclerosis (Ross and Harker, 1976; Shibuya, 1995; Stromblad and Cheresh, 1996). Therefore, the marked inhibition of the proliferation of endothelial cells by baicalein shows promise for the treatment of angiogenic diseases. Recently, baicalein has been reported to induce cell death in human hepatocellular carcinoma cells (Matsuzaki et al., 1996). Based on these findings, baicalein, which can cause inhibition of vascular endothelial cell proliferation and induction of tumor cell death, may have a role in cancer therapy.

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