Baicalin suppresses lung carcinoma and lung metastasis by SOD mimic and HIF-1α inhibition

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ABSTRACT

The dose-related toxicity of anticancer drugs in chemotherapy of clinical carcinoma is the major obstacle to prolonged survival, we want to investigate selective therapeutic efficacy of baicalin on lung carcinoma and explain the basis underlying this phenomenon. In vitro, baicalin inhibited cell proliferation of human lung carcinoma A549 and mouse lewis lung cancer (LLC) in a dose- and time-dependent manner. The inhibitory activity of baicalin against cancer cells was promoted by superoxide dismutase (SOD) addition or hypoxia-inducible factor-1α (HIF-1α) knockdown and was reduced by SOD knockdown but not hypoxia. In vivo, baicalin suppressed tumor growth and prolonged survival in C57BL/6 mice bearing LLC tumor and nude mice bearing A549 carcinoma without systemic toxicity. Further studies showed that baicalin inhibited HIF-1α and enhanced SOD activity without affecting catalase and glutathione-S-transferase (GST) in cancer cells. In addition, baicalin also exhibited a superoxide anion scavenging activity. In conclusion, baicalin could selectively suppress lung carcinoma and lung metastasis by SOD mimic and HIF-1α inhibition.

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1. Introduction

Lung cancer is the first leading cause of cancer death in the United States (Minna et al., 2002). Unfortunately, despite efforts to improve its prognosis, the overall survival of patients with lung cancer is still unsatisfactory (Gautam and Koshkina, 2003). The high mortality rate is mostly due to the high rate of metastasis and severe resistance to both chemotherapy and radiation (Lebedeva et al., 2007). However, therapeutic selectivity of chemical drugs is also the major obstacle to prolonged survival (Workman, 2004). Therefore, development of novel compounds that selectively eliminate tumor cells with minimal toxicity to normal tissues is an important and challenging task, and understanding of the biological differences between normal and cancer cells is essential to achieve this goal.

The development of intratumoral hypoxia and low glucose levels within the tumor cells is a hallmark of rapidly growing solid tumors (Chan et al., 2007). Adaptation of tumor cells to a hypoxic environment results in a resistance to conventional chemotherapy and radiotherapy, and a poor treatment outcome (Chaudary and Hill, 2007). Hypoxia-inducible factor 1 (HIF-1) is the central mediator of cellular responses to hypoxia and plays a central role in tumor progression and angiogenesis in vivo (Gordan and Simon, 2007). In tumor xenograft models, decreased HIF-1 activity is usually associated with a slower growing and less angiogenic tumor phenotype (Melillo, 2006). Histologic analyses have shown that hypoxia-inducible factor-1α (HIF-1α) is overexpressed in many human cancers (Zhong et al., 1999). It is therefore rational to target HIF-1 activity for antitumor intervention.

Another change in cancer cells is the increase in reactive oxygen species generation that plays an important role in maintaining cancer phenotype due to their stimulating effects on cell growth and proliferation (Pelicano et al., 2004). Because of the pro-oxidant status of the cancer cells, they seem to be more susceptible than normal cells to treatment with agents that cause oxidative stress (Laurent et al., 2005). Superoxide dismutase (SOD) can affect tumor cell proliferation via their effects on peroxide levels, overexpression of SOD in human cancer cell lines increases H2O2 production (Zhang et al., 2002). In addition, overexpression of SOD in human cancer cell lines also suppresses hypoxic accumulation of HIF-1α protein under hypoxic conditions (Kaewpila et al., 2008). Therefore, SOD mimic should also reduce tumor growth with minimal affecting normal tissues having higher endogenous antioxidant protein levels. We hypothesized that a single molecule with SOD mimic and HIF-1α inhibitory properties could be beneficial through its ability to enhance the antitumor activity while minimizing systemic toxicity. Baicalin (7-glucuronic acid, 5,6-dihydroxy-flavone) is a flavonoid present in many traditional Chinese medicines. A number of studies show that baicalin has...
anticancer effects and protects against a variety of tissue and organ injuries (Min et al., 2008). In recent studies, baicalin potentiates SOD activity and reduces HIF-1α stabilization (Liu et al., 2008; Cho et al., 2008). In this study, we evaluated the selective therapeutic efficacy of baicalin on lung carcinoma both in vitro and in vivo.

2. Materials and methods

2.1. Materials

Baicalin (high performance liquid chromatographic content >98%) was from Shanzhi Huike Botanical Development Co. (China). RPMI1640 medium was from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS), penicillin, and streptomycin were from Gibco BRL (Rockville, MD). Apoptosis detection kit was from BD Pharmingen (San Diego, CA). 2',7'-dichlorodihydrofluorescein-diacetate (DCHF-DA), dihydroethidium (DHE) and amplex red xanthine oxidase assay kits were purchased from Molecular Probes Inc. (Eugene, OR). SOD (superoxide dismutase), MTT(3-(4,5-dimethyl-2-thiazole)-2,5-diphenyltetrazolium bromide), Xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), Para-hydroxy phenyl acetic acid (pHPA) and horseradish peroxidase were from Sigma Chemicals Co. (St. Louis, MO, USA). MDA (malondialdehyde), SOD, catalase and GST (glutathione-S-transferase) assay kits were from Nanjing jiancheng Bioengineering Institute (China). Anti-HIF-1α and anti-SOD were from BD Biosciences Co. (San Jose, CA). HIF-1α ELISA (enzyme-linked immunosorbent assay) kit was from Wuhan USCN Sciences Co., Ltd (China). A secondary antibody (sheep anti-mouse IgG horseradish peroxidase-linked antibody) was from Sino-American biotechnology Co., Ltd (China). Enhanced chemiluminescence substrate was from ECL Plus (Germany). VEGF (vascular endothelial growth factor), IFN-γ (interferon-gamma) and IL-2,4,10 (interleukin-2,4,10) ELISA kits were from R&D Systems (Minneapolis, MN), siRNAs specific to HIF-1α and to SOD were designed and synthesized by GenePharma Company (Shanghai, China). Ac-DEVD-7-amoно-4-methyl-coumarine (Ac-DEVD-AMC) was from Biosource International Inc (Camarillo, USA). All other reagents were of analytical grade from commercial sources.

2.2. Animal

Pathogen-free female C57BL/6 and athymic nude mice weighing 18–22 g were purchased from Beijing Weitonglihua Animal Co. All mice were maintained in a pathogen-free animal facility for at least 1 week before each experiment. The animal use committee of Pharmaceutical College of Henan University approved all animal study protocols described in this study, and experiments were conducted in compliance with the guide for the care and use of laboratory animals.

2.3. Cell culture

Human lung carcinoma (A549), mouse lewis lung carcinoma (LLC) and mouse embryonic lung fibroblast (1929) cell lines were purchased from American type culture collection (ATCC; Manassas, VA). Cells were cultured in RPMI1640 medium supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. All cells were incubated at 37 °C with 5% CO₂. Hypoxia treatment was performed by placing cells in a modular incubator chamber (Billups-Rothenberg, Del Mar, CA) flushed with a mixture of gas consisting of 1% O₂, 5% CO₂, and 94% N₂.

2.4. Cell proliferation assay

Cells (5 × 10³ cells per well) were plated in 96-well plates and allowed to adhere for overnight. Cells were then treated with baicalin at various concentrations or the vehicle (DMSO, final concentration 0.2%) and incubated under normoxia or hypoxia at 37 °C for 72 h. To observe effects of acetylcyesteine and catalase on baicalin-induced cytotoxicity, cells were cotreated with 10 µmol/l acetylcyesteine or 500 units/ml catalase and baicalin for 72 h. Cell proliferation was examined using MTT assay as previously described (Tang et al., 2007), and the IC50, defined as the concentration of drug required to block 50% cell viability, was determined using SPSS software (version 12.0, SPSS Inc, Chicago, IL). VEGF concentrations were determined in supernatants of tumor cells by ELISA according to standard procedures and corrected for cell number.

2.5. Apoptosis assay

Apoptotic and/or necrotic cells were evaluated by Annexin-V binding and PI uptake using an Annexin V–FITC/PI kit as described by the manufacturer. Briefly, tumor cells were plated at a density of 5 × 10⁵ cells/well into 6-well plates for 24 h. The cells were treated with various concentrations of baicalin and incubated under normoxia or hypoxia at 37 °C for 72 h. The cells were washed with cold PBS and resuspended in Annexin V binding buffer. The cells were stained with Annexin V-FITC for 15 min, washed, and then stained with PI. The samples were analyzed by flow cytometer with CellQuest Pro software. SOD, catalase and GST activities were determined in supernatants of tumor cells according to manufacturer’s instructions and corrected for cell number.

SOD activity is based on the oxidation of NADH mediated by superoxide radical, one unit of SOD activity is defined as the amount of protein required to obtain half-maximal inhibition of NADPH oxidation. Catalase activity is based on the kinetics of 5 mmol/l H₂O₂ degradation at 242 nm, one unit of catalase activity is defined as the quantity that causes reduction of 1 µmol hydrogen peroxide per minute. GST activity is based on the increase in absorbance of the conjugate of GSH and CDNB at 340 nm, one unit of GST activity is defined as the quantity that results in formation of 1 µmol thioether per minute.

2.6. Caspase-3 activity assay

Cells were treated with baicalin as described in apoptosis assay, harvested, and lysed. The cell lysate was centrifuged at 10,000 × g at 4 °C for 30 min and the cytosolic supernatant was incubated with Ac-DEVD-AMC according to manufacturer’s instructions. The amount of AMC was measured at 460 nm using a fluorescence microtiter plate reader, and the data was expressed as nmol AMC per hour per milligram total cellular protein.

2.7. Transfer of siRNA to cells

Cells (2 × 10⁵) were seeded into 6-well plates on the day before transfection. After 24 h (30–40% confluence), the media was replaced with media without serum or antibiotics. The cells were then transfected with the specific siRNA to HIF-1α or SOD using Lipofectamine 2000 reagent (Invitrogen) in accordance with the manufacturer’s instructions. After 5 h, the transfection media was replaced with regular complete medium. After 72 h, cells were harvested or treated as needed for further experiments.

2.8. Cellular reactive oxygen species assay

Cells (1 × 10⁴) were seeded in 48-well plates and incubated for 24 h. The cells were treated with various concentrations of baicalin for 72 h, then were washed with phenol red-free HBSS and loaded with 5 µmol/l DCHF-DA or 10 µmol/l DHE for 40 min at 37 °C. Cellular reactive oxygen species and superoxide anion in 10,000 cells were measured using fluorescence intensity of 2',7'-dichlorodihydrofluorescein (DCF) and ethidium respectively by flow cytometry (Mohanty et al., 1997).
2.9. Extracellular H₂O₂ assay

Cells were treated with baicalin as described in cellular reactive oxygen species assay. Cells were washed with HBSS, resuspended in phenol red-free HBSS containing 6.5 mmol/l glucose, 1 mmol/l HEPES, 6 mmol/l sodium bicarbonate, 1.6 mmol/l pHPA, and 95 µg/ml horseradish peroxidase, and incubated for 1 h. The released H₂O₂ was determined by measuring the dimer formed at excitation and emission wavelengths of 323 and 400 nm (Panus et al., 1993), respectively. The fluorescence intensity was compared with standard concentrations of H₂O₂ determined by absorbance at 240 nm.

2.10. SOD mimic activity assay

SOD mimic activity is measured using a xanthine/xanthine oxidase system which generates superoxide (O²⁻•) and reduces NBT. Briefly, 0.1 ml baicalin was added into 0.5 ml standard reaction mixture containing 0.8 mmol/l xanthine, 0.48 mmol/l NBT, 1 mmol/l EDTA in 10 mmol/l PBS (pH 8.0). After pre-incubation at 37 °C for 5 min, the reaction was initiated by adding 1 ml xanthine oxidase (0.05 U/ml). After 20 min incubation, the reaction was stopped by the addition of 2 ml SDS (69 mmol/l). The absorbance at 560 nm was measured by spectro-photometer (Bio-Rad) and the blank value was subtracted from each sample.

In addition, the rate of H₂O₂ production was also measured in the amplex red xanthine/xanthine oxidase assay system using amplex red xanthine/xanthine oxidase assay kit as described by the manufacturer.

2.11. Western blot analysis

Whole-cell extracts were prepared from baicalin treated or untreated cells cultured in 6 well plates. After incubation, cells were harvested and resuspended in lysis buffer, washed with ice-cold PBS and lysed in extraction buffer (40 mmol/l Tris–HCl, pH 7.5, 150 mmol/l KCl, 1 mmol/l EDTA, 1% Triton X-100, 100 mmol/l NaVO₃, 1 mmol/l PMSF) supplemented with the protease inhibitor cocktail. The protein (50 µg) was separated on 10% SDS-PAGE and transferred onto a PVDF membrane (Roth, Karlsruhe, Germany). The membranes were blocked with 5% non-fat milk in Tris-buffered saline (TBS) at 4 °C, and then incubated with mouse anti-HIF-1α monoclonal antibody (1:500), mouse anti-SOD monoclonal antibody (1:300), or mouse anti-β-actin in TBS containing 5% non-fat milk for 1 h at room temperature. Horseradish peroxidase-linked anti-mouse IgG (1:1000) was used as a secondary antibody (in TBS containing 5% non-fat milk for 30 min at room temperature), and antigen–antibody complexes were detected using an enhanced chemiluminescence kit (ECL Plus, Amersham, Freiburg, Germany).

2.12. In vivo antitumor activity

C57BL/6 mice and athymic nude mice were subcutaneously injected in the right flank with 2 × 10⁵ LLC cells and 5 × 10⁶ A549 cells, respectively. The mice were kept in a pathogen-free environment and treated twice weekly. When the tumors reached a size of 200–400 mm³ (10 days for LLC cells and 30 days for A549 cells following implantation), mice were randomized into one of three experimental groups (15 each group for C57BL/6 mice and 12 each group for nude mice) and treatment was initiated according to the indicated schedule/dose. Baicalin (100 mg/kg) or vehicle, formulated in 0.5% CMC-Na, was administered daily by oral gavage for 4 weeks; vinorelbine (5 mg/kg), formulated in 0.9% NaCl, was injected intravenously on days 1, 8 and 15 after grouping. Tumors were measured with calipers twice weekly, and tumor volume was calculated using the following formula: tumor volume (mm³) = [(width)²×length]/2. Treatment-related toxicity was determined by mouse weights weekly.

Five C57BL/6 mice or four nude mice in each group were sacrificed at days 28 after treatment. Tumors were dissected and weighed. Lungs were collected and evaluated for metastases. Serum cytokines including IFN-γ, IL-2, IL-4, IL-10 were determined by ELISA according to manufacturer’s instructions. Tissue MDA, antioxidant enzymes in serum and tissues including SOD, catalase and GST were examined using commercial kits. VEGF and HIF-1α in tissues were measured by ELISA as described by the manufacturer. The total number of hematopoietic cells in bone marrow and leukocytes from blood were counted using a Zeiss microscope. The remaining mice in each group were used for survival analysis. Kaplan–Meier plots were constructed to show the percentage animals remaining in the study as a function of time following treatment. Statistical significance between the treated versus control groups was evaluated by log-rank analysis using SPSS software.

The tissue samples of tumor and lung were prepared as described previously (Choi et al., 2006). Briefly, fresh tissue was frozen in liquid nitrogen and chopped. 0.2 g tissue was resuspended in 1 ml lysis buffer (10 mmol/l HEPES, pH 7.6, 2 mmol/l DTT, 1 mmol/l Na₂VO₄, 100 mmol/l NaF, 0.4 mmol/l PMSF, 0.1 mmol/l EGTA, 10 mmol/l Na₃P₂O₇, 1× protease inhibitor cocktail) for 15 min on ice. After centrifugation at 8000 g for 15 min, supernatants were used as tissue extracts. The pellet was resuspended in 300 µl lysis buffer (20 mmol/l HEPES, pH 7.5, 400 mmol/l NaCl, 10 mmol/l NaF, 10 mmol/l PNPP, 1 mmol/l Na₂VO₄, 0.1 mmol/l EDTA, 10 mmol/l Na₂MoO₄, 10 mmol/l β-glycerophosphate, 20% glycerol, 1 ml/l DTT, and 1× protease inhibitor cocktail) for 30 min at 4 °C, then centrifuged at 13,000 × g for 30 min, the supernatants were used as nuclear extracts.

2.13. Statistical analysis

The results were expressed as mean ± S.E.M., statistical analysis was performed using one-way ANOVA, followed by a post-hoc Tukey test or Student’s t test. P < 0.05 was considered as statistically significant.

3. Results

3.1. Baicalin exhibits preferential activity against lung cancer cells

Baicalin, a common ingredient in many traditional Chinese medicines, has been demonstrated to own the antioxidant and anticancer functions. To observe whether baicalin has a striking preferential activity against the transformed cells, lung cancer cells and lung fibroblasts were treated with different concentrations (1–200 µmol/l) of baicalin for 24–72 h. As shown in Fig. 1A–C, baicalin significantly inhibited cell viability in lung cancer cells in a dose- and time-dependent manner. Its IC₅₀ values were 20.79 µM (95% CI 17.09 to 25.45 µM) in A549 cells and 30.53 µM (95% CI 25.76 to 36.51 µM) in LLC cells, respectively, following 72 h drug exposure. In contrast, baicalin had little toxicity against the normal lung fibroblasts as measured by MTT assay. Consistently, baicalin induced apoptosis in A549 and LLC cells but not in lung fibroblasts tested by Annexin-V binding and PI uptake (Fig. 1D and E). Cell apoptosis was further confirmed by measurement of caspase 3 activity using Ac-DEVD-AMC as a substrate (Fig. 2). However, antioxidant acetylcysteine or the H₂O₂ scavenger catalase could suppress baicalin-induced cytotoxicity in tumor cells (Supplement Fig. 2).

3.2. Effects of baicalin on SOD activity and HIF-1α accumulation

In previous studies, baicalin affects cellular SOD and HIF-1α (Liu et al., 2008; Cho et al., 2008). To evaluate whether preferential activity of baicalin is related with SOD activity and HIF-1α accumulation, cells were treated as described in text. As shown in Fig. 3A and B, baicalin enhanced SOD activity without affecting catalase and GST in lung cancer cells and lung fibroblasts. Consistently, baicalin increased cellular...
reactive oxygen species and reduced superoxide anion in lung cancer cells after treatment with baicalin (Fig. 3C–E). Interestingly, baicalin didn't significantly enhance reactive oxygen species in lung fibroblasts although it also decreased superoxide anion (Fig. 3C–E), mainly likely due to low basal reactive oxygen species output and high catalase activity in normal cells (Fig. 3B–E). Extracellular H$_2$O$_2$ assay confirmed these results (Fig. 3F). However, there was significant difference between lung cancer cells and lung fibroblasts in their HIF-1$\alpha$ accumulation in response to baicalin. Baicalin inhibited HIF-1$\alpha$ in lung cancer cells but increased its accumulation in lung fibroblasts (Fig. 4A), this was further confirmed by VEGF, the major angiogenic factor induced markedly by HIF-1$\alpha$ (Fig. 4B). In addition, baicalin displayed a SOD mimic activity determined by NBT reduction and H$_2$O$_2$ production in xanthine/xanthine oxidase system (Fig. 4C and D), its IC$_{50}$ value for inhibition of NBT reduction was 1.20 $\mu$M (95% CI 0.63 to 2.51 $\mu$M).

3.3. Effects of SOD or HIF-1$\alpha$ on baicalin activity

Based on the above observations, we also investigated effects of SOD or HIF-1$\alpha$ on baicalin activity by siRNA interference (Supplement Fig. 1). HIF-1$\alpha$ induction and SOD addition. As shown in Fig. 5A and B, the preferential activity of baicalin against lung cancer cells was abrogated by HIF-1$\alpha$ siRNA but not HIF-1$\alpha$ accumulation induced by hypoxia although both of them inhibited cell viability. In contrast, SOD siRNA did not affect cell viability but suppressed growth-inhibitory activity of baicalin in lung cancer cells (Fig. 5C), whereas SOD addition had similar effect on cells as baicalin and amplified inhibitory effect of baicalin on lung cancer cells (Fig. 5D).

3.4. Baicalin exhibits significant therapeutic activity against lung cancer in vivo

To observe whether baicalin has the same preferential activity in vivo as in vitro, we explored the antitumor activity of baicalin using an implant model of LLC mouse lung cancer and a xenograft models of
A549 human lung cancer. In both models, vinorelbine, which is currently used as first line therapy for human lung cancer, was used as a comparison for baicalin activity. As shown in Fig. 6, mice treated with baicalin or vinorelbine had statistically significantly smaller tumors than untreated mice at days 7, 14, 21, and 28 following initiation of treatment (Fig. 6A-C). Without drug treatment, the tumor-bearing C57BL/6 mice and nude mice died within 2 months and 3 months respectively. The median survival times for baicalin-treated C57BL/6 mice and nude mice were 53 days (95% CI 47 to 59 days, \(P<0.01\)) and 101 days (95% CI 86 to 116 days, \(P<0.01\)) respectively versus 39 days (95% CI 37 to 41 days) and 80 days (95% CI 72 to 88 days) for corresponding untreated mice (Fig. 6D,E). In contrast, treatment with vinorelbine did not significantly increase the median survival times in both mice (\(P>0.05\)).

Implantation of LLC tumor cells into mice results in the development of metastatic node in lung. To extend our observations, the effect of baicalin on metastasis was also explored. The number of metastatic nodes significantly decreased after baicalin treatment, the incidence of lung metastasis for baicalin-treated C57BL/6 mice was 50\% versus 100\% for untreated mice at the end of study. By contrast, mice treated with vinorelbine developed similar lung metastasis to untreated mice, likely due to systemic toxicity (Fig. 6F).

3.5. Baicalin has little toxicity for normal tissues

Conventional chemotherapy for patients with cancer often damages normal tissues. To evaluate whether baicalin results in systemic toxicity, vinorelbine was used as a comparison. Hematopoietic system was investigated by blood leukocytes and bone marrow cells. Immune system was examined by serum cytokines including the type 1 cytokines IFN-\(\gamma\) and IL-2, and the type 2 cytokines IL-4 and IL-10, which governed T-cell homeostasis by...
balance between immunostimulation of the former and immuno-suppression of the latter (Clerici et al., 1997). In addition, we checked body weight loss in mice bearing tumor. As shown in Fig. 7A–D, in

linied with preferential activity against lung cancer, baicalin had no effect on blood leukocytes, bone marrow cells and 4 serum cytokines, and did not cause body weight loss in treated C57BL/6 mice. In

Fig. 4. Baicalin has different effects on HIF-1α accumulation and mimics SOD activity. (A) Baicalin inhibited HIF-1α accumulation in LLC cells and increased it in L929 cells tested by Western blot. (B) Baicalin had similar effects on VEGF to HIF-1α accumulation in lung cancer cells and lung fibroblasts. (C) and (D) Baicalin inhibited NBT reduction and enhanced the rate of H2O2 production in Xanthine/XOD system. Data excluding Western blot are presented as mean ± S.E.M., n = 3. Two asterisks indicate a significant difference from control without baicalin application (P<0.01).

Fig. 5. Effects of ether SOD or HIF-1α on preferential activity of baicalin, cells were treated with baicalin for 72 h under different condition and examined by MTT assay. (A) Effect of HIF-1α siRNA on activity of baicalin. (B) Effect of HIF-1α induced by hypoxia on activity of baicalin. (C) Effect of SOD siRNA on activity of baicalin. (D) Effect of SOD addition on activity of baicalin. Data are presented as mean ± S.E.M., n = 3.
contrast, vinorelbine significantly reduced this index. Similar results were obtained in nude mice.

3.6. The effects on SOD and HIF-1α of Baicalin are similar in vivo and in vitro

To investigate whether baicalin has similar effects on SOD and HIF-1α in vivo as in vitro, the activities of anti-oxidative enzymes including SOD, catalase and GST in serum, tumor and lung were examined by commercial kits. The levels of HIF-1α and VEGF in tumor and lung were tested by ELISA. In addition, MDA contents in tumor and lung were used to confirm the preferential activity of baicalin against lung cancer. As shown in Fig. 8A–D, baicalin enhanced SOD activity but had no effect on activities of catalase and GST in both mice bearing-tumor. Interestingly, baicalin reduced accumulations of cellular and nuclear HIF-1α in tumor but increased them in lung (Fig. 9A,B), similar effect of baicalin on VEGF was obtained in both tissues (Fig. 9C). Importantly, baicalin increased MDA content in tumor but decreased it in lung (Fig. 9D), which exhibited preferential activity of baicalin against tumors.

4. Discussion

An ideal anticancer agent should be toxic to malignant cells with minimum toxicity in normal cells. However, almost all artificial agents currently being used in cancer therapy are known to be toxic and produce severe damage to normal cells (Moreira et al., 2001). Natural products derived from plants have recently received much attention as potential chemopreventive and chemotherapeutic agents, such as resveratrol and naringenin (Middleton et al., 2000). Baicalin is a very promising flavonoid for cancer prevention and treatment in preclinical models (Wang et al., 2008), however, therapeutic selectivity and the molecular mechanisms underlying cytotoxicity of baicalin in cancer cells remain unclear. Because baicalin is a major component of Scutellaria baicalensis, which has long been extensively used in Chinese herbal medicine as an anti-inflammatory agent for treatment of bronchitis and asthma (Kubo et al., 1984), we compared the cytotoxic effect of baicalin in lung cancer cells and lung fibroblasts. Interestingly, baicalin exhibited a preferential activity against lung cancer cells but had little effect on lung fibroblasts, which led us to explore the mechanisms underlying cytotoxicity of baicalin and its activity in vivo.
The biochemical difference between normal and cancer cells constitutes a basis to selectively kill cancer cells. In previous studies, several SOD mimic agents and HIF-1α inhibitors showed a relatively higher selection toward the tumor cells for their killing effects (Barik et al., 2007; Chau et al., 2005). We hypothesized that a compound with SOD mimic and HIF-1α inhibitory properties will provide a better anticancer effect. In this study, baicalin exhibited direct and indirect SOD activity by NBT reduction and reactive oxygen species production in lung cancer cells. Importantly, baicalin reduced HIF-1α accumulation in lung cancer cells but increased it in lung fibroblasts, this could be related to the notion that transformed cells and normal cells may have different resting levels of reactive oxygen species and antioxidant capacity (Schumacker, 2006). siRNA interference showed that SOD siRNA did not affect cell viability but suppressed growth-inhibitory activity of baicalin in lung cancer cells, whereas HIF-1α siRNA abrogated preferential activity of baicalin against lung cancer.
cells. These results suggest that SOD activity of baicalin will benefit to enhance its therapeutic efficacy and the opposing effects of baicalin on HIF-1α in normal and cancer cells will increase its therapeutic index. We further confirmed the therapeutic efficacy and the mechanisms underlying cytotoxicity of baicalin using both tumor models in vivo. Our study showed that baicalin has a superior selectivity compared to vinorelbine, a common drug for lung cancer treatment.

The intracellular redox environment could be determined by the relative rate of production and removal of reactive oxygen species. The SODs dismutate O$_2^-$ into H$_2$O$_2$, whereas the catalases and peroxidases convert H$_2$O$_2$. The previous studies showed that maintaining a slightly elevated intracellular concentration of O$_2^-$-inhibited apoptotic signaling (Pervaiz et al., 2001), whereas decrease in intracellular superoxide sensitizes bcl-2-overexpressing tumor cells to receptor and drug-induced apoptosis (Clement et al., 2003). Considering the fact that overexpression of SOD have shown tumor suppression in various cancer cell types (Oberley, 2001), SOD could either inhibit or promote the process of carcinogenesis depending upon its expression level. In a recent finding, the effect of intracellular reactive oxygen species on oncogenesis is dependent on the ratio of intracellular superoxide to H$_2$O$_2$ in that a predominant increase in superoxide supports cell survival and promotes oncogenesis whereas a tilt in favor of H$_2$O$_2$ prevents carcinogenesis by facilitating cell death signaling (Pervaiz and Clement, 2007). In our studies, baicalin exhibited direct and indirect SOD activity but had no effect on catalase and peroxidase, whereas antioxidant acetylcysteine or the H$_2$O$_2$ scavenger catalase could suppressed baicalin-induced cytotoxicity in tumor cells, this was consistent with excessive H$_2$O$_2$ production of baicalin and baicalin-induced apoptosis. However, it is possible that the redox status of other cancer cells is different from lung cancer cells, and baicalin may not be sufficiently potent in selective killing in some cancer cells.

In conclusion, our study suggests that a single molecule with SOD mimic and HIF-1α inhibitory properties can be designed to selectively target tumor tissues while having minimal systemic toxicity. The ability to preferentially kill malignant cells is a promising feature of baicalin, this property of baicalin is able to be further explored for the development of antitumor agents with a higher therapeutic index.

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Appendix A. Supplementary data


References


