Anticancer Properties of *Scutellaria baicalensis* Root in Aspect of Innate Immunity Regulation

Przeciwnowotworowa aktywność tarczycy bajkalskiej w świetle regulacji wrodzonej odporności

Abstract

Cancer disease is accompanied by inflammation, oxidative stress, strong proliferation of cancer cells with growth of tumor and its vascularisation, metastases and also by reduction of innate immunity. Anticancer effect of *Scutellaria baicalensis* flavones in view of present literature data is discussed. Inhibition of growth different tumor cell lines by flavones isolated from root of the plant: wogonin, baicalein and baicalin, but not wogonoside is presented. The anticancer activity of the flavones is connected with reduction of high level reactive oxygen species (ROS), reduction of inflammatory reaction, and NFκB activation. Inhibition of cell cycle and involvement cyclins in the process is discussed. The most important anticancer reaction of *Scutellaria* flavones is stimulation of apoptosis of cancer cells. Molecular mechanism of anti-prostate, anti-breast cancer activity of *Scutellaria baicalensis* extract and herbal mixture PC-SPES is discussed. Role of flavonoids obtained from *Scutellaria* extracts in human leukemia cell lines are described. Importance of leukocyte antiviral innate immunity for chemotherapy and course of acute leukemia disease is presented. Strengthening of the leukocyte antiviral resistance by flavones of *Scutellaria baicalensis* and modulation of cytokines production seem also very important in cancer therapy (Adv Clin Exp Med 2010, 19, 4, 419–428).

Key words: anticancer properties, *Scutellaria baicalensis* flavones, reduction of inflammation, inhibition of cell cycle, stimulation of apoptosis, effect on innate immunity.
Two main problems of present chemotherapy that play a major role in treatment of tumor patients include: toxicity of drugs toward normal tissue and development of resistance of the tumor cells toward apoptosis. Thus, it is an urgent requirement to improve cancer therapy. Minimizing side-effects and maximizing efficacy became a major goal in the development of apoptosis inducers.

Traditional Chinese remedies have been recently recognized as a new source of anticancer drugs (inducers of apoptosis) and new chemotherapy adjuvant to enhance the efficacy of the therapy and to ameliorate its side effects. However, their healing mechanisms are still not completely known.

Scutellaria baicalensis Radix (common name: Huang-Qin in China, and Ougon in Japan), and its flavonoid compounds as: baicalin, baicalein, wogonin, oroxylin and others, is one of the most popular, and multi-purpose remedy used in China, Japan and in several oriental countries. In Traditional Chinese Medicine (TCM) extracts from the roots obtained from various species of genus Scutellaria, especially Scutellaria baicalensis Georgi, are widely used for clinical treatment of hyperlipidaemia, atherosclerosis, hypertension, dysentery, common cold, viral, bacterial and inflammatory disease (esp. atopic dermatitis) and others [1].

Apart from above properties, Scutellaria alone, or in combination with other herbs, has been recently shown to possess cytostatic effect on several cancer cell lines in vitro [1–3] and also in vivo in mouse tumor models [4, 5]. The low toxicity to non-malignant cells has made Scutellaria baicalensis very attractive as a new anticancer drug.

The Bioactive Constituents of Scutellaria Baicalensis Radix

The major constituents of Scutellaria baicalensis radix (Scutellaria baicalensis Georgi) are flavonoids, chiefly baicalin (up to 14%), baicalein (up to 5%), wogonin (0.7%) and wogonin-7-O-glucoronide (wogonoside, 4.0%). To identify the antitumor components of Scutellaria sp., many studies have been carried out using purified compounds. Antitumor constituents of these species, especially Scutellaria baicalensis Georgi, are flavones: baicalin (baicalein 7-O-glucuronide), baicalein (5,6,7-trihydroxyflavone), wogonoside (wogonin 7-O-glucuronide), and wogonin (5,7-dihydroxy-8-methoxyflavone) with ratios to the dry material about 10.11%, 5.41%, 1.3% and 3.55% respectively.

Among the flavones wogonin, baicalein and baicalin have been shown to inhibit growth of various human cancer cell lines. The doses of 50% inhibition of tumor proliferation are ranging between 20 and 200 μM, depending on the types of tumor cells tested. In addition, these compounds also possess a direct cytotoxicity to a large panel of human malignant cell lines by inducing apoptotic cell death.

Molecular Mechanisms of Antitumor Activity of Scutellaria Baicalensis Radix Flavonoids

Molecular mechanisms anticancer activity of flavonoids are diverse and include: anti- and pro-oxidative effects, cell cycle inhibition, apoptosis induction, inhibition of intracellular signaling by transcription factors (i.e. NFκB), anti-inflammatory and also anti-viral activities.

Effect of Flavonoids on ROS Scavenging

ROS (Reactive Oxygen Species) play an important role in defense against pathogens and in regulation of diverse cellular function, including intracellular signaling. High levels of ROS induce oxidative stress that can lead to different diseases, including cancer. Flavonoids belong to the phenolic antioxidants (PhOH) capable of transferring electron free radicals and also to act as chelators of redox – active metal ions. The anti- and pro-oxidative properties of flavonoids from Scutellaria are excellent described by Li-Weber [5]. The author writes that the flavonoids interfere with the oxidation of lipids and other molecules by the rapid donation of hydrogen atom to radicals (ROO⁺ + PhOH = ROOH + PhO.), and take the redox-active metal ions away. Unlike the hydroxyl (•OH) and superoxide (ROO⁻) radicals which are highly active due to a very short half-life (esp. hyroxyl), the phenoxy radical intermediates are relatively stable so they do not initiate (propagate) further radical reactions and thereby stop the radical reaction chain. Thus, the association of flavonoids intake with reduced risks of cancer can be largely explained by the anti-ROS activities of these compounds. Investigation of the bioactivity and structure relationships of different flavonoids has revealed that the antioxidant properties of flavonoids involves the presence of 2,3-unsaturation in conjugation with a 4-oxo group in the C-ring, the hydroxyl groups in
the B-ring and the 5-hydroxy group in the A-ring. The main flavones of *Scutellaria baicalensis* as baicalin, baicalein and wogonin possess the 2,3-unsaturation and the 4-cko in the C-ring and the rather rare hydroxylation in plant flavonoids (at C5, C6, C7) in the A-ring, and they provide a high antioxidant and anti-radicals properties [7].

The free-radical scavenging activities of baicalein and baicalin were investigated by Shieh at al. [7]. Both flavonoids exhibited capacity to eliminate the superoxide radical, respectively: bajkaline: 7.31 × 10^4 μ/g; bajkalin: 1.19 × 10^5 μ/g. However they did not have significant effect on scavenging hydroxyl radical. It was supposed that antioxidant activity of baicalin is based on the superoxide radical elimination and baicalein is a pleasing xanthine oxidase inhibitor.

Among the examined flavonoids, wogonin at the highest pitch affects inhibition of nitric oxide production, while oroxylin A inhibits lipid peroxidation in the most potent way [6].

### Pro-oxidative Activity of Flavonoids

Several studies showed that flavonoids have not only antioxidative, but also prooxidative activity [6–8]. It was demonstrated that peroxidase catalyzes one-electron oxidation of the phenolic ring of etoposide (an anticancer compound) and/or interaction of this phenolic moiety with reactive radicals to yield its phenoxy radical. Thereby, etoposide acted as an effective radical scavenger. The peroxidase-catalyzed phenoxy radicals, although did not lead to oxidation of phospholipids (suggesting that the radicals were not reactive enough to trigger lipid oxidation), were shown to contribute pro-oxidant effects via oxidation of glutathione (GSH) and protein thiols in the redox-cycling. The evidence for the pro-oxidant activity of flavonoid phenoxy radicals was shown by their ability to deplete gluthathione (GSH) of tumor cells. Wogonin, baicalein and baicalin were all shown to cause depletion of GSH content in human hepatoma cell lines and leukemia cell line therefore it is believed that the anticancer activity of these compounds may also involve a pro-oxidant mechanism.

### Inhibition of NF-κB Activation by Flavones

NF-κB is an inducible transcription factor that controls the expression of over one hundred genes involved in immunity, inflammation, proliferation, defense against apoptosis and also ROS. By activation certain intracellular signaling cascades including NFκB, may contribute to tumor development. NF-κB is often found to be constitutively or over-activated in malignant cells. Currently, activation NF-κB is considered to be involved in a survival and activation expression of various anti-apoptotic genes e.g. Bcl-2, Bcl-XL, Mcl-1 that promote survival of many types of tumors. Tumor cells are also often shown to produce increased levels of ROS due largely to the increased metabolic activity and to mitochondrial malfunction.

Reduced NF-κB activity was observed in myeloma cell lines after baikalein treatment. Recent studies have shown that wogonin can scavenge.0_{2} and shifts the cellular redox potential to a more reduced state H_{2}O_{2} and thereby down-regulate ROS-mediated NF-κB activation [10]. In most tumor cell lines wogonin, and baikalein suppressed the expression of genes BcI-2, BcI-X, and Mcl-1 [6].

### Cell Cycle Inhibition by Flavones

The cell cycle consists of 4 phases: M, G1, S, G2. M phase (mitosis) is usually followed by cytokinesis. During interphase, which involves G1, S, G2 phase, a cell grows and replicates its genetic material (DNA). During G1 phase (the interval between mitosis and S phase) succeed RNA and peptides synthesis, in S phase succeed DNA replication and in phase G2 (the interval between S phase and mitosis) supergene repair of damaged DNA and preparation the cell for mitosis.

*S. baicalensis* through cell cycle regulation manages cancer proliferation what has been proved in many experiments. One can distinguish a few checkpoints in cell cycle according to the type of tumors where flavonoids impact is perceptible. Cyclins are one of the regulatory molecules which determine a cell’s progress through the cell cycle.

Significant decrease in cyclin D1 protein level in wogonin treated breast cancer cell lines was affirmed. Cyclin D1 expression was also suppressed in baikalein treated prostate cancer cell lines. However only in baikalein-treated lung cancer cell lines a decrease in both cyclins D1 and B1 levels was noticed. In flavonoids-treated leukemia cell lines the cell cycle was inhibited at G2/M phase [9].

*Scutellaria baicalensis* caused significant G0-G1 phase arrest with concurrent decrease in S phase in prostate carcinoma cell line. However, baikalein caused a significant G2-M phase arrest and decrease in G0-G1 phase simultaneously [10].

In the case of wogonin or baikalein treated prostate and other cancer cell lines, the percentage of cells in G1 phase increased while in S phase decreased [10, 11]. Data show that myeloid leukemia
cells, lymphocytic leukemia cells, lymphoma cell lines myeloma cell lines treated with S. baicalensis also decreased in S phase and had an increased number of cells in the sub-G1 population.

**Apoptosis Induction**

It is well-established that anticancer agents induce apoptosis (programmed cell death), which is necessary to keep cellular homeostasis and normal development. Apoptosis induction is one of the major desired effects of anticancer therapy. Proapoptotic baicalin mechanism probably is related with mitochondrial pathway activation. Mechanism of baicalin-induced cytotoxicity in leukemia Jurkat cells was examined. Cells showed characteristic morphologic change of apoptosis. Ueda et al. [12] have shown that baicalin induced cytochrome C release into cytosol, disruption of mitochondrial transmembrane potential, then caspase-3 activation and consequently nuclear degradation. The results indicate that apoptosis by baicalin is mediated by mitochondrial pathway. Baicalein also induced apoptosis, which was confirmed by DNA condensation and fragmentation. In cells H 460-treated baicalein affirmed decreasing in Bcl-2, increasing in caspase-3 activity and p53 and Bax protein levels. It has been known that p53, which is a tumor suppressor protein, regulate the Bcl-2 and Bax protein expression.

**Anti-Inflammatory Effect in Flavonoids of Scutellaria Baicalensis Radix in Possible Oncological Therapy**

Chronic inflammation may contribute to cancer development. Flavonoids wogonin, baicalein and baicalin of Scutellaria sp. have been shown to protect tissues from inflammation in vitro and in vivo in various animal models [13–16].

The anti-inflammatory effect of the Scutellaria flavones is in part due to their inhibition of cytokines production [17] and also nitric oxide (NO) production via down-regulation of several inflammation-associated genes such as inducible NO synthase (iNOS), cyclooxygenases (COX) and lipoxygenases. NO, a highly reactive free radical, and its synthase (NOS) are ubiquitous in malignant tumors and are known to exert both pro- and antitumor effects [18–21]. Interaction between NO and p53 is a crucial pathway in inflammatory-mediated carcinogenesis.

Flavonoids wogonin, baicalein and baicalin were shown to inhibit inducible synthase (iNOS) gene expression and reduce NO production in vitro and in vivo in animal models [22]. The pro-inflammatory product of elevated cyclooxygenase-2 (COX-2) activity, prostaglandin E2 (PGE2), plays direct role in malignant progression of most solid tumors, including colon, breast, head and neck, uterus, and stomach carcinomas. Two enzymes COX-1 and COX-2 participate in the biosynthetic pathway of PGE2. Increased levels of COX-2, represents the best target for therapeutic intervention. Administration of the non-steroid and anti-inflammatory drugs or the COX-2 inhibitors has been shown to reduce the overall number and size of adenomas in patients and also in experimental animal models [18, 23, 24].

Scutellaria baicalensis extracts were shown to suppress PGE2 synthesis and inhibit COX-2 expression in vitro in two head and neck tumor cell lines and inhibit head and neck tumor growth in vivo in mice.

It was also reported that wogonin, but not baicalein and baicalin inhibited lipopolysaccharide (LPS)-induced COX-2 gene expressions in murine macrophages [25]. Wogonin was also shown to reduce COX-2 expression from murine skin fibroblast NIH/3T3 cells, interleukin – 1β or TNFα and from human lung epithelial cancer cells [18]. The effect of wogonin on COX-2 was also examined in animal experiments which showed that it potently lowered mRNA levels of COX-2 in a sub-chronic and also in an acute skin inflammation model.

Chemokines and chemokine receptors participate in inflammation and infection by stimulation of cell migration. Baicalein could inhibit binding of a number of chemokines to human leukocytes. This event led to reduction of chemokines to human leukocytes. It was shown by Li et al. [26] that baicalin did not directly compete with chemokines for binding to receptors, but rather acted through its selective binding to chemokines of the CXC (stromal cell-derived factor-1α, IL-8), CC (macrophage inflammatory protein-1β, monocyte chemotactic protein-2) and C (lymphotactin) subfamilies but not to the CX3C chemokine fractalkine/neurotactin or other cytokines, such as TNF-α and IFN-γ. Whether other flavones of Scutellaria sp. are also capable to bind chemokines to limit their biological function needs to be clarified.

**Inhibition of Carcinogenic Viruses by Scutellaria baicalensis Flavonoids**

The antiviral effect of Scutellaria baicalensis flavones was described by the present authors previously [27]. Here is presented antiviral activity against viruses which have been identified as car-
cinogens. Among them six viruses are cancerous for human: human papilomaviruses (HPVs) type 16 and 18, Epstein-Barr virus (EBV), two hepatotropic viruses hepatitis B virus (HBV), hepatitis C virus (HCV), lymphotropic human T-cell virus type 1 (HTLV-1) and human immunodeficiency virus type-1 (HIV-1). HTLV-1, and HIV-1 are oncogenic retroviruses. HTLV-1 may cause adult T-cell leukemia/lymphoma (ATLS) in small part of carriers of the virus.

Several types of cancer have been strongly associated with the HIV-1 infection and developing acquired immunodeficiency syndrome (AIDS). Kaposi sarcoma, lymphoproliferative disorders and cervical cancer are observed in AIDS patients. HBV and HCV cause malignant disease of liver, hepatocarcinoma. EBV infection is associated with lymphoid and solid tissue malignancies including nosopharyngeal carcinoma, non-Hodgkins lymphoma T cells and Hodgkin lymphoma. Reverse transcriptase is a target for baicalin and baicalein inhibition of retroviruses replication in leukocytes [28, 29]. Sometimes, however, other stages of viral replication i.e. viral entry or release or HIV-1 protease is inhibited by flavones [30–32]. Thus, flavones have been suggested by some investigators as potential anti-retroviruses agents.

Hepatocellular carcinoma (HCC) is one of the most frequent and malignant diseases worldwide. The major HCC risk factors are chronic HBV and HCV virus infections that attribute to HCC development in more than 80% of the HCC cases worldwide.

Effect of wogonin on HBV replication was studied in vitro in two human cell lines: MS-G2, HBV-producing cell line, and HepG2.2.15, HBV-transfected cells [33]. In both, suppression of virus was observed. In vivo suppression effect of wogonin was observed in duck infected with DHBV (duck hepatitis B virus) [34].

Another study aimed at seeking effective drugs against HCV screened 20 Chinese herbs and revealed five herbs, including Scutellaria baicalensis with significant inhibitory effects on replication of HCV-RNA [35, 36].

Japanese authors Konoshima et al. [37] investigated 14 flavones isolated from Scutellaria baicalensis for their inhibitory effects on the EBV early antigen (EBV-EA) activation. Among the flavones investigated 5,7, 2'-trihydroxy- and 5,7, 2, 3-tetrahydroxyflavone showed noticeable inhibitory effects on the EBV-EA activation. These flavones were also shown to inhibit skin tumor promotion in a two-stage carcinogenesis test in vivo in the mouse.

Antiviral activity of different agents (natural plant or synthetic) may be expressed by their virucidal effect, inhibition of viral replication in infected cells, stimulation antiviral nonspecific innate or specific immunity. The data presented here concerned the effect of flavones from Scutellaria baicalensis on replication of oncogenic viruses. The structure, taxonomic affiliation and mode of replication of the viruses are quite different. The results of flavones effect on replication of single virus are not repeatable. All the obtained results suggest that another mechanism, common for the different viruses might be engaged.

As it was suggested previously [27], the unspecified resistance of peripheral blood leukocytes (PBL) to viral infection maintained in vivo seems to fulfill requirement of the common mechanism against viruses. Flavones from Scutellaria were found to strengthen the resistance [17]. The leukocyte resistance is very important for course of not only viral, but also cancer disease. Acute leukemia is an example of cancer disease. Course of the disease and successive chemotherapy was found to be dependent on degree of PBL resistance [38]. Effective chemotherapy was obtained only in patients with resistant PBL. All patients with resistant PBL survived, while those with deficiency of resistance died in relatively short time. Zitvogel and Kroemer [39] in review on anticancer immunotherapy present the possibility of participation some adjuvants in the therapy. The adjuvant, however, must have direct cytotoxic effect on cancer, anti-inflammatory effects and influence innate immunity. As the flavones isolated from Scutellaria express all the activities, they may be classified as adjuvant. Possible mode of the antitumor effect by adjuvant proposed by Zitvogel and Kroemer presented in their publication indicate possible direct and indirect immune-mediated anticancer effects.

Molecular Mechanism of Anti-Prostate Cancer Activity of Scutellaria baicalensis Extract and Herbal Mixture PC-SPES

Management of prostate cancer that has spread beyond the capsule is a difficult problem. Innovative and non-toxic approaches to the disease are urgently required. Recently, a commercially available Chinese herbal medicine called PC-SPES showed potent antitumor activities on a variety of malignant cells in vitro and in vivo in patients with advanced stages of the disease [6, 20, 40–42].

PC-SPES is a herbal mixture: Chrysanthemum indicum L. (syn. Dendranthema morifolium L. Des
Moul), Panax pseudo-ginseng Wall., Glycyrrhiza uralensis Fisch, Rabdosia rubescens Hara, Scutellaria baicalensis Georgi, Ganoderma lucidum Karst, Isatis indigotica Fort, Serenoa repens Hook.

used by prostate cancer patients as an alternative form of treatment. Since PC-SPES is derived from eight individual herbs, each with distinct as well as overlapping properties, it was interesting to investigate whether a particular herb in the formulation principally accounts for the biological properties of PC-SPES. Hsieh TC and Wu JM [40] tested the ability of extracts from individual herbs of PC-SPES on proliferation and prostate specific gene expression in androgen-dependent LNCaP cells, and synergism and antagonism among individual herbal constituents of PC-SPES was observed. Chen at al. [20] have isolated a few of the most active compounds of ethanol extract of PC-SPES. Among them, baicalin was the most abundant (about 6%) in the ethanol extract.

Recently, Adams et al. [41] investigated the interactions (the additive or synergistic effects) of botanical extract combinations of PC-SPES against the viability of prostate cancer cell lines. The most active extracts: S. baicalensis, D. morifolium, G. uralensis and R. rubescens were tested as two-extract combinations. S. baicalensis and D. morifolium when combined were additive with a trend toward synergy, D. morifolium and R. rubescens together were additive. The remaining two-extracts combinations showed antagonism. The four extracts together were significantly more effective than the two-by-two combinations and the individual extracts alone.

PC-SPES was evaluated for its ability to inhibit clonal growth, and to induce cell cycle arrest of three human prostate cancer cell lines (LNCaP, PC-3, and DU 145). Western blot analysis examined the effect of PC-SPES on levels of p21(waf1), p27 (kip 1), Bcl-2 and E-cadherin in the three cell lines, and telomerase activity was examined by telomeric repeat amplification protocol (TRAP) assay. Furthermore, the effect of oral administration PC-SPES (250 mg/kg/day) on growth of PC-3 and DU 145 tumors present in male BNX nu/nu triple immunodeficient mice was studied (LNCaP cells were not analyzed in mice because they grow only with difficulty in these immunodeficient mice) [42]. In results authors stated that PC-SPES markedly inhibited clonal growth of LNCaP, PC-3 and DU 145 prostate cancer cells, with a 50% inhibition (ED50) at approximately 2 μl/ml. Pulse-exposure studies showed that a 5-day pulse-exposure to PC-SPES (2 μl/ml) in liquid culture achieved a 50% inhibition of PC-3 clonal growth in soft agar, suggesting that the growth inhibition mediated by the extracts remained after removal of PC-SPES. Cell cycle analysis using the prostate cancer cell lines found that PC-SPES induced a significant increase in the number of cells in G0-G1 and G2-M, with a concomitant decrease in the number of cells in 5 phase. PC-SPES (2 microl/ml, 4 days) increased slightly the levels of p21(waf1) in the three cell lines, decreases the levels of Bcl-2 in PC-3 by 40%, and the levels p27(kip 1) and E-cadherin and telomerase were unchanged in each of the lines. In vivo treatment with oral PC-SPES of male BNX mice having DU 145 tumors produced significant inhibition of their growth (p < 0.001), with no objective side effects including blood chemistries, weights, or autopsy analysis. The PC-SPES showed no statistical effect on the in vivo growth of PC-3 cells [42].

Molecular mechanisms of anti-prostate cancer activity of Scutellaria baicalensis extracts was studied by Ye et al., too [43]. 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), cyclins/cdk5 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 IC 50 (50% growth inhibition concentration) were slightly more sensitive than LNCaP (IC 50 = 0.15 mg/ml), although the former is androgen-independent, S. baicalensis was more effective in inhibition of cell growth compared with PC SPES (IC 50 = 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 [40].

Scutellaria baicalensis also inhibited prostate-specific antigen production in LNCaP cells. Finally, S. baicalensis suppressed expression of cyclin D 1 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 [43].

In other study Ikezoe et al. [44] stated that baicalin is a major component of PC-SPES (6% of the total ethanolic extracts of PC-SPES) which inhibits the proliferation of human cancer cells via apoptosis and cell cycle arrest. Baicalin was evaluated for its ability to inhibit clonal growth, and
to induce cell cycle arrest of various cancer types prostate cancer cell lines (PC-3, DU145, LNCaP), MCF-7 breast cancer cell line, HL-60 myeloblastic leukemia cell line, and NB4 promyelocytic leukemia cell line. The ability of baicalin to induce apoptosis of cancer cells was examined by both staining with Annexin V and detection of cleavage of Poly (ADP-ribose) polymerase (PARP). Western blot analysis examined the effect of baicalin on levels of p21 (waf1) and p27 (kip1) in those cells. Furthermore, induction of differentiation in HL-60 cells was measured by expression of CD 11b. In results, baicalin inhibited the clonal proliferation of LNCaP and PC-3 prostate cancer cell lines, and the HL-60 and NB4 myeloblastic/promyelocytic leukemia cell lines with a 50% inhibition (ED 50) that ranged between 6.4 × 10^(-6) to 12 × 10^(-6) mol/L. Cell cycle analysis showed that baicalin (2 × 10^(-5) mol/L, 4 days) caused a G(0), G(1) and G(2)/M accumulation of LNCaP and HL-60 cells, respectively. Concomitantly, differentiation and apoptosis were induced in HL-60 cells, as measured by expression of CD 11b antigen, staining with Annexin V, and detection of cleavage of PARP. Moreover, baicalin enhanced the expression of the cyclin-dependent kinase inhibitor, p27 (kip1) in LNCaP and HL-60 cells. In results, baicalin may be a novel adjunctive therapy for selected malignancies including prostate cancer, as authors suggested [44].

The Role of Flavonoids of Scutellaria baicalensis in Human Breast Cancer Cells

Baicalein inhibited the proliferation of estrogen receptor – positive human breast cancer MCF-7 cells in vitro, with median effective concentration 5.3 μg/ml [45].

Recently, baicalin was investigated for tumor cell-specific cytotoxicity, apoptosis inducing activity and signal pathway against the MDA-MB-231 human breast cancer cell line [46]. After the MDA-MB-231 cells had been treated with baicalin, the dead cells and apoptosis was detected. The effects of baicalin on the levels of reactive oxygen species (ROS, Ca(2+)) and mitochondrial membrane potential on MDA-MB-231 cells were examined by flow cytometric assays. The ROS caused endoplasmic reticulum (ER) stress, confirmed by the increase of GADD153 and GRP78 in the examined cells (confirmed by confocal laser microscopy examination) and indicated that both proteins were translocated to the nucleus.

The effects of baicalein on the expression of apoptotic-regulated genes, such as Bcl-2 family and caspase, were detected by Western blotting. The further investigate the apoptotic pathway and the role of Ca(2+) induced by baicalein, a caspase-3 inhibitor and Ca(2+) chelator were used to block caspase-3 activity and Ca(2+) in MDA-MB-231 cells. In conclusion authors stated that baicalin induced apoptosis via Ca(2+) production, mitochondria-dependent and caspase-3 activation in MDA-MB-231 cells [46].

Recently, Japanese authors Murashima et al. [47] demonstrated that the proliferation of estrogen-responsive mouse Leydig tumor cell line B-1Fis induced via suppression of 5-lipoxygenase activity followed by decrease of leukotrienes (LTs). Additionally, it has been reported that LTD4 induces apoptosis in B-1F cells. In this study, authors examined effects of Saiboku-to (a traditional Japanese medicine KAMPO), which has suppressive activities for LT production and release, on the proliferation. Saiboku-to promoted, but Scutellaria baicalensis (one of components of Saiboku-to), significantly inhibited the proliferation of B-1F cells in vitro and in vivo.

Role of Flavonoids of Scutellariae baicalensis Extracts in Human Leukemia Cell Lines

Chemotherapy agents, particularly those that can induce apoptosis, are the major intervening strategy in the treatment of leukemia. Among them, baicalin was found to induce apoptosis in human promyelocytic leukemia HL-60 cells through multiple pathways and may be interesting strategy in treatment of leukemia. Recently, Huang et al. [48] studied the crude aqueous extract of Scutellaria baicalensis which flavones were responsible for the cytotoxic effect on HL-60 cells. Authors observed a dose-dependent reduction in cell viability when cells were with either wogonin or aqueous extract of S. baicalensis. Several of the apoptotic features including deoxyribonucleic acid (DNA) fragmentation and increased caspase-3 activity were found in cells treated with wogonin or S. baicalensis extract. The changes were associated with down-regulation of Bcl-2 and not Bax. Furthermore, treatment of HL-60 cells with wogonin or S. baicalensis extract led to the inhibition of human telomerase reverse transcriptase (hTERT), human telomerase-associated protein 1 (hTP1) and c-myc messenger ribonucleic acid (m-RNA) expression.
Authors suggested that the inhibition of HL-60 cell growth is mediated partly through the induction of Bax/Bcl-2 apoptosis and by telomerase inhibition through suppression of c-myc, which is a promoter of hTERT. All these activities of *Scutellaria baicalensis* extracts on leukemia cells are important in chemopreventive effects shown recently by Wang et al. [49].

References


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