Come Back to Root – Therapeutic Activities of Scutellaria baicalensis Root in Aspect of Innate Immunity Regulation – Part I

Abstract
Scutellaria baicalensis Georgi (Engl. skullcap or baical skullcap; Chin huang qin, Jap. ougon or ogon) is widely used in traditional and modern oriental medicine. This Asian plant is acclimated and cultivated in Central European condition (including Poland). Therapeutic activities of flavonoids of skullcap roots are known. Among the flavones: baicalein, baicalin, wogonin and wogonoside are the most important. In the review, several potential activities of the extracts and flavones of Scutellaria baicalensis roots are described. Anti-bacterial, anti-viral, anti-tumor, anti-inflammatory activities in aspect of innate immunity regulation seems very important. Moreover, possible therapeutic activities in neurodegenerative diseases promise their therapeutic usefulness. Many diseases, i.e. tumor, neurodegenerative, include Alzheimer disease (AD), and inflammatory diseases (hepatitis, colitis, arthritis, dermatitis and others) are accompanied by inflammatory reactions. The inflammation of neurones is often the result of over-activation of immunity (i.e. by β-amyloid in AD). Some diseases are accompanied by deficiency of innate immunity reactions. The deficiency was described for infectious (bacterial and viral) and tumor diseases. In view of the wide activities of Scutellaria flavonoids in different inflammatory diseases, regulatory effect on the innate immunity is postulated (Adv Clin Exp Med 2008, 17, 3, 337–345).

Key words: Scutellaria baicalensis flavonoids, antiinflammatory activity, antimicrobial, antitumor, innate immunity.

Streszczenie

Słowa kluczowe: flavonoidy tarczycy bajkalskiej, aktywność przeciwpalna, przeciwdrobnoustrojowa, przeciwnowotworowa, wrodzona odporność.
The title of the review was inspired by Carl Nathan article [1]. According to Nathan’s, history of medicine (given by Anonymus) start in the ancient time.

- 2000 BC – Here, eat this root.
- AD 1000 – That root is heathen. Here, say this prayer.
- 1850 – That prayer is superstition. Here, drink this potion.
- 1920 – That potion is snake oil. Here, swallow this pill.
- 1945 – That pill is ineffective. Here, take this penicillin.
- 1955 – Oops… bugs mutated. Here, take this tetracycline.
- 1960 – 1999 – 39 more “oops”… Here, take this antibiotic.
- 1999 – 2000 – The bugs have won! Here, eat this potion.

Scutellaria baicalensis – History, Characteristics, Therapeutic Activities

Scutellaria baicalensis Georgi (Engl. skullcap or Baikal skullcap, Chin. huang qin, jap. wogon or ogon, Korean hwang-geum) is a perennial herb of Lamiaceae family, cultivated in China, Japan, Korea and listed in the Chinese, Japanese and Korean Pharmacopoeias and others. The dried roots of this plant – Radix Scutellariae (Skullcap Root) has a particular high flavonoid content (over 25%), which gives it a yellow colour and gives rise to its traditional name of Golden root or Golden skullcap. Multiple biological properties have been described for this herb, including anti-inflammatory, antiviral, anticarcinogenic, free-radical-scavenging, antioxidant and immunostimulatory effects. Radix Scutellariae baicalensis is widely used in traditional and modern oriental medicine as remedies for fever, allergic diseases and inflammation, especially against bacterial and viral infections of the respiratory and the gastrointestinal tract, i.e. chronic bronchitis, bacillary dysentery, viral hepatitis, and acute biliary tract infection, pancreatitis, colitis and others; also, in dermatological diseases, as remedies for fever, allergic diseases and inflammatory, antiviral, anticarcinogenic, free-radical-scavenging, antioxidant and immunostimulatory effects.

Antimicrobial Activity of Scutellaria baicalensis Root
Anti-Bacterial Activity

The root of S. baicalensis has a wide antibacterial activity in vitro against hemolytic streptococcus, pneumococcus, meningococcus, Staphylococcus aureus, Corynebacterium diphtheriae, Shigella dysenteriae, Bacillus anthracis, Salmonella typhi, Salmonella paratyphi, Proteus, Escherichia coli, Pseudomonas aeruginosa, Bordetella pertussis and Vibrio comma. Baicalin and baicalein are the major antibacterial active component. In tests with selected oral bacteria, including suspected periodontopathogens: Bacteroides melanogenicus intermedius was found to be most sensitive to a 2% decoction of S. baicalensis [2, 3]. A hot aqueous extract of the roots inhibited the growth of Alcaligenes calcoaceticus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus at concentrations of 200.0–400.0 µg/ml [4].

The antibacterial activity of Scutellaria baicalensis flavonoids is especially important in
the case of bacterial resistance to antibiotics. The resistance is a serious global problem and includes strains of beta-lactam-resistant *Staphylococcus aureus*. Baicalin for example, showed synergy with beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA). Japanese and Chinese authors [5, 6] noted also the remarkable synergies between baicalein and tetra-cycline, and baicalein and beta-lactams against methicillin-resistant *Staphylococcus aureus* (MRSA). Minimum inhibitory concentrations (MICs) of benzylpenicillin against MRSA was reduced when 10 µg mL(-1) baicalein was added. The obtained reduction was from 125 and 250 µg mL(-1) to 4 and 15 µg mL(-1), respectively. This activity of baicalein or baicalin was dose-dependent. From the other authors constated, that the killing of MRSA and beta-lactam-resistant *S. aureus* cells by 10 to 50 µg mL(-1) ampicillin, amoxycillin, benzylpenicillin, methicillin and cefotaxime was potentiated by 25 µg mL (-1) baicalin. They concluded that baicalin has the potential to restore the effectiveness of beta-lactam antibiotics against MRSA and other strains of beta-lactam-resistant *S. aureus*. In view of this activity, plant complementary medicine for treatment bacterial and viral diseases is an alternative.

**Anti-Fungal Activity**

The aqueous extract was shown in vitro to be active against *Candida albicans* and *Rhodotorula rubra* and against 10 kinds of skin fungi including *Trichophyton violaceum*, *Microsporum audouini* and *Microsporum canis* [3, 7]. The extracts of *Scutellaria baicalensis* roots obtained by the successive exhaustion in chloroform and in ethyl acetate present clear fungistatic activities in vitro against to some cutaneous and ungual pathogenic fungi, and particularly upon strains of *Candida albicans*, *Cryptococcus neoformans* and *Pityrosporum ovale*. The ethyl acetate extract appears the most interesting because of its efficiency and of its stability. An antifungal substance is found out as the baicalein which possessed potent specific activity against the pathogenic yeasts with MIC of 70–100 µg/ml. The good inhibitory activity of baicalein could be linked to the group hydroxyl (-OH) in position seven of the molecule (ring A).

**Anti-Viral Activity**

Complementary medicines might be useful in therapy of such threatening viral diseases as AIDS, hepatitis C and B, or even influenza and herpes, especially herpes genitalis. Studies in vitro show inhibition effect of *Scutellaria baicalensis* flavones on human immunodeficiency virus (HIV) replication [8, 9, 10]. Positive effects were obtained after treatment HIV-infected patients with the herbal decoction, or isolated flavones. Inhibition of viral replication was found for infections with hepatitis type C virus (HCV) [11], hepatitis type B virus (HBV) [12], influenza A and B viruses[13], herpesviruses type 1 and 2 (HSV-1, HSV-2) and Epstein-Barr virus (EBV) [14]. All the viruses differ in their virions structure (RNA and DNA, with or without envelope), taxonomic affiliation, and replication process. HIV for example, has RNA containing genome, surface envelope and belongs to Retroviridae family, influenza viruses (Orthomyxoviridae) have genomic RNA and envelope, HCV (Flaviviridae) is RNA containing virus without envelope, HBV (Hepadnaviridae) and HSV-1, HSV-2, EBV belonging to Herpesviridae contain genomic DNA and envelope. The process of replication is different for each of the viruses. There are several literature data concerning inhibitory activity of Scutellaria flavones on HIV replication. The data are, however, insufficient and usually unrepeatable. According to the data, baicalin may inhibit HIV-1 replication at the level of viral entry to cell, or on the level synthesis of viral enzymes: reverse transcriptase, or protease [8–10]. It must be stressed that extracts of *Scutellaria baicalensis* roots might exert beneficial effect on anti-HIV HAART therapy. Protease inhibitors used in the therapy causes significant gastrointestinal disturbances such as nausea. According to Aung et al. [15] *Scutellaria baicalensis* decreases ritonavir induced nausea. Extremely large number of different viruses inhibited by skullcap flavones is astounding and suggest more universal mechanism engaged in the antiviral effect.

**Effect of *Scutellaria baicalensis* Flavones on Inflammatory Reactions**

Chronic inflammatory reactions lead to development some inflammatory diseases (i.e. IBD – inflammatory bowel disease), or may accompany numerous illness as: infectious, tumor, allergic, autoimmunologic, neurodegenerative diseases. Therefore the ability of skullcap flavones to soften or modulation of the inflammatory reactions look promising in the respect of their therapeutic usefulness. Inflammatory reactions include production and activity cytokines, chemokines, adhesion and other molecules as C-reactive protein (CRP),
nitric oxide (NO). In production most of them and their activity transcription factor NFκB is involved. After activation, NFκB translocate to nucleus and bind to promoters of cytokine and other proteins genes and activate production or action. Tang et al [16] studied effect of wogonin, flavonoid of Scutellaria baikalensis, on the NK cells activity, macrophage phagocytosis, release of cytokines: IL-1α, TNFα, and NFκB activation in leukocytes from rats. They found that wogonin promoted the activity NK cells and macrophages and induced cytotoxicity. Wogonin stimulated also IL-1α and TNFα level in dose-dependent way. Kim et al [17] studied the effect of baicalin on age-related NFκB activation in kidney tissue from old rats feded with baicalin. Increased expression of NFκB targeting genes (hemoxygenase-1, inducible nitric oxide synthase (iNOS) and COX-2 in old rats was reduced by baicalin. Furthermore, baicalin pretreatment decreased tissue myeloperoxidase activity and lipid peroxidation, but increased the superoxide dismutase level. In vitro studies indicated that the beneficial effect of baicalin was associated with reduced cytokine production from lymphocytes and reduced TNFα-induced hepatocyte apoptosis. These results suggest that baicalin has therapeutic potential for T-cell-mediated liver injury [18].

Interleukin-12 (IL-12) is an important cytokine for maintenance of normal systemic defense and bioregulation. The Japanese herbal medicine Sho-saiko-to (TJ-9) containing Radix Scutellariae and Radix Glycyrrhizae uralensis has been administered to 1.5 million Japanese patients with chronic liver diseases. TJ-9 is known to significantly suppress cancer development in the liver and has macrobiotic effects. Effects of the Japanese herbal medicine “Sho-saiko-to” (TJ-9) on interleukin-12 production in patients with HCV-positive liver cirrhosis was studied. The levels of IL-12 produced by the patients were significantly lower than those produced by healthy subjects (p < 0.01, p < 0.05). However, when TJ-9 was added to the cultures, the IL-12 production levels in both cell fractions increased approximately three fold, and the levels from the monocyte/macrophage fraction were almost the same as those from healthy subjects. This effect of TJ-9 was attributable to two of its seven herb components, that is Scutellaria root and Glycyrrhiza root. One possible mechanism for the macrobiotic effects of TJ-9 on liver-cirrhosis patients may be the improvement in IL-12 production.

Chemokines belong to a large family of inflammatory cytokines responsible for migration and accumulation of leukocytes at inflammatory sites. Baicalin possess anti-inflammatory activity through its ability to complex with chemokine IL-8 and thus reduces their capacity to bind and activate their receptors. Woo et al [19] found that balcalin has capacity to inhibit cyclooxygenase (COX-2) gene expression in macrophage cell line Raw 264. In view of the effect of scutellaria flavones on inflammatory reactions the therapeutic activity in cardiacl, dermatologic, hepatitis and pancreatitis is possible. The further experiments seem to confirm the idea. Since oxidants play also roles in inflammatory diseases, the effect of extract from Scutellaria baikalensis (SbE) on cultured cardiomyocyte death caused by oxidant stress in the context of explanation the mechanism of lethal cell damage in ischemia was studied by Chang et al [20]. They found that SbE attenuate oxidant stress and protect the myocytes from lethal oxidant. In vivo study on therapeutic efficacy of baicalin in experimental severe acute pancreatitis (SAP) of rats was performed by group of Chinese scientists Zhang et al. [21]. The contents of amyrase, TNFα, IL-6, malondialdehyde and PLA(2) were significant lower in the treated than in control group, what indicate significant therapeutic effect of baicalin on SAP rats. Next the pathological changes in multiple organs pancreas, liver, kidney, and lung in SAP rats were studied. Zhang et al found them milder than those in control group. The experimental works performed on rats indicate possible therapeutic effect in pancreatitis.

A new “medical food” recently hit the American pharmaceutical market as a treatment for osteoarthitis (OA). LIMBREL (flavocoxid) consists of a propriety blend of the two types of flavonoids: baicalin from Scutellaria baikalensis root and catechine from Acacia catechu bark. These ingredients are Generally Recognized AS Safe (GRAS) by U.S. Food and Drug Administration (FDA), and are listed as such by regulation in Volume 21 Code of Federal Regulations (CFR), Section 182, 184, and 186. LIMBREL® – named flavocoxid (flavonoids having antiinflammatory activities), its capsules (U.S. pending) by oral administration, for the clinical dietary management of the metabolic processes of osteoarthritis (OA). According to FDA instruction the preparation must be administered under physician supervision.

Metabolic processes are important in the progression of OA. After initial damage to the joint due to trauma, overuse of the joint, or genetic factors, a cascade of inflammation triggered by the release of cytokines (e.g. TNF-α, IL-β, IL-6) begins the development of OA. These cytokines up-regulate the increased expression of COX-2 (cyclooxygenase-2) and 5-LOX (5-lipooxygenase) enzymes, which metabolize fatty acids in the joint.
This process is both enzymatic as well as oxidative, and occurs at a cellular level the essential fatty acid – arachidonic acid (AA) is converted into various inflammatory products. In addition, AA is converted via an oxidative mechanism mediated by ROS to F2-isoprostane, malondialdehyde, and 4-hydroxynonenal, oxidized lipids which directly degrade cartilage and induce other inflammatory proteins. With age, elevated levels of AA accumulate both from the diet, and from the increased conversion of phospholipids produced by further damage to cells in the joint. Though the initial damage that causes OA is mainly due to overuse, injury, or genetics, the cartilage degradation which occurs over time is characterized by the chronic, heightened metabolism of the accumulated AA to excess inflammatory metabolites. Therefore, OA is sustained by imbalanced AA metabolism. Managing AA metabolism benefits OA patients by decreasing the damaging, metabolic inflammatory processes in the joint to improve functional mobility, reduce stiffness and decrease joint discomfort.

In treatment of OA, a mixed extract containing two naturally occurring flavonoids: baikalin from *Scutellaria baicalensis* and catechin (derived of flavones) from *Accacia catechu* was tested for COX-1 and COX-2, and 5-LOX inhibition, via enzyme, cellular, and in vivo models [22]. The 50% inhibitory concentration for inhibition of both ovine COX-1 and COX-2 peroxidase enzyme activities was 15 microg/ml, while the mixed extract showed a value for potato 5-LOX enzyme activity of 25 microg/ml. Prostaglandin E2 generation was inhibited by the mixed extract in human osteosarcoma cells expressing COX-2, while leukotriene production was inhibited in both human cell lines, immortalizes THP-1 monocyte and HT-29 colorectal adenocarcinoma. In an arachidonic acid-induced mouse ear swelling model, the extract decreased edema in a dose-dependent manner. When arachidonic acid was injected directly into the intra-articular space of mouse ankle joints, the mixed extract abated the swelling and restored function in a rotary drum walking model. These results suggest that this natural, flavonoid mixture baikalin with catechin acts via “duals inhibition” of COX and LOX enzymes to reduce production of pro-inflammatory eicosanoids and attenuate edema in an in vivo model of inflammation.

**Antitumor Effect of Scutellaria baicalensis Flavonoids**

Traditional Chinese herbal medicines (TMC) that for centuries have been used in disease prevention and treatment are finding use as alternatives to Western cancer therapies. PC-SPES is herbal mixture used by prostate cancer patients as an alternative form of treatment. *Scutellaria baicalensis* and two other herbs (*Glycyrrhiza uralensis* and *Serenoa repens*) were found to lower intracellular and secreted prostate-specific antigen (PSA). From a panel of the herbas, the purified plant flavonoids from *Scutellaria* roots were examined for anticancer activity. Cancer is hyperproliferative disorder that involves morphological cellular transformation, dysregulation of apoptosis, uncontrolled cellular proliferation, invasion, angiogenesis and metastasis. In vitro mechanism of PS-SPES according to Chen [23] include the following: induction of apoptosis and cell cycle modulation, inhibition of cell proliferation, down-regulation of bcl-2, bcl-6, effect on cell nuclear antigen and prostate-specific, antigen proteins, downregulation of androgen receptor and upregulation of p53, bax and p21 proteins.

Antiproliferative effect on various cancer cell lines was shown by different groups of scientist. Chen et al. [23] demonstrated significant suppression of prostate cancer cell growth by restriction of cell cycle progression at G(1)/S and drastic reduction in expression of androgen receptor and PSA. The antiproliferative effect was obtained in human hepatocellular carcinoma cell lines and in lung cancer cell line by some authors [24–26]. It must be stressed that the growth inhibitory effect of flavonoids was observed among human cancer cells but not in normal diploid cells [24]. Inhibition cell proliferation is not the only anticancer mechanism of flavones.

Sun JP et al. [27] investigated the effect of baikalin on insulinoma (tumour in the islets of Langerhanse) cell line and the molecular mechanism involved. After treatment with baikalin, the number of cells in mitotic stage and survival rate of cells obviously decreased, and cell proliferation was inhibited in a drug concentration and acting time-dependent manner with the appearance of apoptotic insulinoma cells. During the apoptotic process, the activity of caspase-3 was elevated by baikalin in time-dependent manner. With the increase of the concentration of baikalin, the number of cells in S-phase obviously decreased from 38.2% to 9.4%, while the percentage of cells in GO/G1 phase increased from 56.4% to 85.9%, indicating cells were arrested in G1-phase. Meanwhile, the activity of cyclin reduced remarkably. In conclusion authors stated that baikalin could induce apoptosis of insulinoma cells, which might be correlated with the activity of caspase-3, and inhibiting proliferation of insulinoma cells in a concentration- and time-dependent manner, in which the action of baikalin in down-regulating...
the gene transcription and expression of cyclin may play an important role. Anticancer, antiradical and antioxidative actions of novel Antoksyd S® and its major components, baicalin and baicailein was found by Ciesielska et al. [28, 29]. Antiproliferative and apoptic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines by Scutellaria baicalensis flavonoids was found by Kumagai et al. [29]. Additionally antimutagenic and antiradical properties of flavones from the roots of Scutellaria baicalensis (Antoksyd®) was found by Woźniak et al [30].

**Effect of Scutellaria baicalensis Flavonoids in Neurodegenerative Diseases**

Beneficial effect of Scutellaria baicalensis root was observed in neurodegenerative diseases such as Alzheimer disease, ischemia, spinal cord injury, multiple sclerosis, prion diseases, Parkinson disease, and epilepsy [32–39]. These diseases are caused by neuro-immunomechanisms, which contribute actively to the neurodestructive process. Besides them, the most of the literature data concern mechanism of explanation neuroprotection by flavones of Scutellariae radix and its antioxidative activities.

Reactive oxygen species (ROS), nitric oxide (NO) and product of arachidonic acid metabolism prostaglandin E2 (PGE2) are important mediators in a number of neurodegenerative diseases. Effects flavones of skullcap on release of these mediators is frequently studied.

In Alzheimer disease, amyloid β (Aβ) protein is known to increase free radical production and lipid peroxidation leading to apoptosis neurons. Heo et al. [37] found that baikalein and baicalin reduced cytotoxicity of Aβ protein in PC12 nerve cells. Li et al. [38] showed that baikalein by anti-inflammatory and antioxidant properties protect primary midbrain rat neuron-glia cultures from degeneration. Effect of amelioration of total flavonoids from stem and leaves of Scutellaria baicalensis Georgi on cognitive deficits, neural damage and free radicals disorder induced by cerebral ischemia in rats was found by Shang et al. [39]. Potent inhibitory effect of flavonoids in Scutellaria baicalensis amyloid beta protein-induced neurotoxicity was found by Heo et al. [37].

The capacity of the S. baicalensis extract and baicalin for reducing the effect of psychoemotion-al stress on the blood system is apparently related to their influence on the adrenergic structures and confirm the adaptogenic activities of drugs.

**Effects of Scutellaria baicalensis on Innate Immune Reactions**

Beneficial effects of Scutellaria baicalensis extracts was observed in astounding wide spectrum of illnesses as: infectious, tumor, inflammatory, autoimmune, neurodegenerative, asthmatic and others. The wide spectrum of activities prompted authors to search for common mechanism engaged in development of all the diseases. Such common reaction attending all the diseases is inflammation. Inflammatory reaction results from overstimulation cells of immune system especially innate system, and defective natural regulation based on adenosine, SOCS and Tyro-3 molecules or cytokines IL-10, TGFβ [40]. Among different ideas, the regulation of innate immunity by Scutellaria baicalensis root seemed to be the most probably. Among different receptors, the toll-like (TLRs) are the most important receptors of innate immune system. They play a fundamental role in recognizing infectious and noxious agents as well as products of tissue damage. TLRs are capable of initiating both protective and damaging inflammatory responses. Reactions of the innate immunity include: phagocytosis, the production and activity cytokines, chemokines, and adhesion molecules and other proteins, the killing of infected or changed cells by apoptosis, NK cells and complement activated by natural lectins or alternatively and the cytokine-dependent resistance of leucocyte and other cells ex vivo to viral infections [41].

The last activity was studied in our laboratory and the test based on vesicular stomatitis virus (VSV) infection of peripheral blood leukocytes was elaborated All these mentioned mechanisms are engaged in maintaining of innate immunity. The innate immunity is regulated by the natural mechanisms functioning in healthy organism. Deficiency of this immunity is accompanied by frequent bacterial and viral infections and development of tumor diseases [42, 43]. What is interesting, that presence of resistancy of leukocytes from blood of patients with acute leukemia is associated with remission of healthy cells after chemotherapy and much longer survival time of the patients. According to Rifkin et al. [44] defective or delayed clearance of apoptic bodies might result in the release of autoantigens. This defect may be a primary effective cause of the development of autoimmunity. Small amounts of cytokines (IFNs and TNFα) produced by specialized cells participate in maintaining innate immunity, however, the cytokines may be directed against the host. Baccala and colleagues [45] con-
sider IFNs type I and type II as the most pathogenic effectors in autoimmunity. A higher production of cytokines, oxidative stress and high production of NO accompany autoimmunity and neurodegeneration. In central nervous system (CNS) microglia cells presenting TLRs, play a role of innate immunity cells, which protect neurons from neurotoxic effects. However, when they are over-stimulated (i.e. by Aβ in Alzheimer disease) instead protect, they kill neurons.

In the context of the antiviral activity of *Scutellaria baicalensis*, against broad spectrum viruses, the effect of baicalein- and wogonin-enriched preparations on three mechanisms of innate immunity: natural resistance of human leukocytes to viral infection, production of cytokines and killing virally infected leukocytes were studied.

The obtained results indicate that extracts containing baicalein and wogonin express antiviral activity by augmentation of the resistance/innate antiviral immunity of PBLs sensitive to infection, by stimulation of killing the virally infected leukocytes and by modulation of cytokines production i.e. inhibition IFNα, IFNγ an stimulation TNFα, IL-12 and IL-10 (46).

Strengthen of antiviral resistance of leukocytes, stimulation of IL-12 production, killing virally infected cells by flavonones from *Scutellaria baicalensis* radix may be the mechanisms of their antiviral and perhaps an antitumor activity.

Several biotechnology and pharmaceutical companies have programmes to develop new drugs that are either: agonists of TLRs to enhance immune responses against tumors and infectious diseases or to correct allergic responses; or antagonist designed to reduce inflammation due infection or autoimmune and neurodegenerative diseases. Flavonones from *Scutellaria baicalensis* root with regulatory effect on innate immunity, have opportunity to join the new drugs list.

### References


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