Pharmacological Effects of Scutellarin, An Active Component of Genus Scutellaria and Erigeron: A Systematic Review

Sebastian Chledzik, Jakub Strawa, Katarzyna Matuszek and Jolanta Nazaruk
Department of Pharmacognosy
Medical University of Bialystok, Bialystok, Poland

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Abstract: Flavonoid compound scutellarin (Scu) is quite frequently met in the plant kingdom, particularly in the genus Scutellaria (Lamiaceae) and Erigeron (Asteraceae). The extract of the herb of Erigeron breviscapus, containing this component in high amount, has been used for many years in traditional Chinese medicine. In recent years, studies have made great progress on the usefulness of Scu for treating various diseases by testing its mechanism of action. They support the traditional use of Scu rich plant in heart and cerebral ischemia. Scu can potentially be applied in Alzheimer’s disease, Helicobacter pylori infection, vascular complications of diabetes and as an inhibitor of certain carcinomas. Various methods were designed to improve its isolation from plant material, solubility, absorption and bioavailability. On the basis of recent studies, it is suggested that Scu could be a promising candidate for new natural drug and deserves particular attention in further research and development.

Keywords: Scutellarin; Flavone Glucuronide; Therapeutic Potential; Pharmacokinetic Studies; Review.

Introduction

Traditional Chinese medicine (TCM) since ancient times has utilized a large arsenal of natural products which nowadays are regarded as potential sources for new drugs. One of such plants is Erigeron breviscapus (Vant.) Hand-Mazz. from the family Asteraceae. The herb (Deng-Zhan-Xi-Xin) is used for the treatment of various diseases such as heart conditions and pain (Gao et al., 2007a). Breviscapine, a purified flavonoid extract from this species containing no less than 85% of scutellarin (Scu), is commercially available and is...
administrated for cerebral infarctions (Xia et al., 2007). Among many medical claims attributed to this extract is anti-oxidant potential, which was evaluated using various assays (Wang et al., 2008). The herb of *Erigeron breviscapus* is a component of Deng-yan granule. The preparation is used for the treatment of coronary heart disease (Zhang et al., 2014). The TCM recommends injection of the extract of *E. breviscapus* for treating hypertension. The investigations showed that the components of this formula might reduce perivascular type I collagen, improve vascular compliance and reverse myocardial, interstitial and vascular remodeling through the inhibition of protein kinase C (Xiong et al., 2013).

Scu is the main compound that is critical for testing the quality and efficiency of *E. breviscapus* for clinical usage. According to Chinese Pharmacopoeia the source must be standardized on the content of this compound (Zhang et al., 2014).

Chemically Scu is a flavonoid compound, glucuronide of scutellarein (5,6,7,4'-tetra-hydroxyflavone-7-O-β-glucuronide) (Fig. 1). It is found not only in the genus *Erigeron* (Qu et al., 2001), but also it is widely distributed in the genus *Scutellaria* (Malikov and Yuldashev, 2002) and in *Conyza* (Chai et al., 2008), *Centaurea* (Formisano et al., 2012) or *Anaphalis* (Hua and Wang, 2004).

The research on Scu has become very intensive. In recent years studies investigating the mechanism of action of Scu in order to treat various diseases have made great progress. The purpose of this paper is to review the pharmacological possibilities of Scu. We also presented current knowledge on the pharmacokinetics of the compound and the methods, which can be used to improve its isolation from plant material and its bioavailability.

**Literature Search and Inclusion Criteria**

The main electronic data bases used in the literature search were Scopus and Web of Knowledge. The search terms were “Scu activity” and “Scu pharmacokinetic”. The articles that were chosen were limited to ones published in English. Appropriate papers were picked out manually. Those with irrelevant research directions or a low level of evidence were excluded.
The Results of the Literature Search

The electronic search for the end of September 2017 of Scopus and Web of Science Core Collection for a key word “Scu activity” identified a total of 196 and 101 potentially relevant articles, respectively. On the basis of the amount of the work, we chose the most often described types of the activity which are presented below.

Biological Activity of Scutellarin

Cardiovascular and Cerebrovascular Diseases

Scu at the doses ranging from 50 to 200 mg in TCM clinics is used in the treatment of coronary heart disease, angina pectoris, myocardial ischemia, stroke, and cerebral thrombotic diseases (Yang et al., 2009). In the animal model, the compound (in doses 15 mg/kg and 50 mg/kg) significantly reduced the myocardial infraction, inhibited myocardium cells apoptosis, and focal brain ischemia (Lin et al., 2007). Scu curbed the size of cerebral infacts, improved neurological deficits and reduced the permeability of the blood–brain barrier after cerebral ischemia/reperfusion (Hu et al., 2005). Pharmacological studies suggest that the cardio- and neuroprotective effects of Scu depend on the up-regulation of the endothelial nitric oxide synthase (eNOS) expression (Hu et al., 2005; Yang et al., 2009) and the down-regulation of the VEGF, bFGF, and iNOS expression. It is essential, since eNOS produces nitric oxide (NO) with beneficial effects in ischemic brain injury, while inducible isoform (iNOS)-derived NO is detrimental during ischemia (Hu et al., 2005). It is well known that eNOS-produced NO contributes to vasodilation, platelet aggregation impairment and reduces endothelial adhesiveness for monocytes (Li et al., 2009).

Another possible mechanism for the protective activity of Scu on cardiomyocytes during ischemia reperfusion is based on the suppression of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, and IL-8), creatinine kinase release and the anti-oxidant properties of the compound (Wang et al., 2016b). Moreover, in vivo (rats) studies showed that thanks to this last activity, Scu in dose 5 mg/kg/day (i.v.) can attenuate doxorubicin toxicity in cardiac tissue (Sun et al., 2017).

Scu induces an endothelium-independent vasorelaxant effect through the inhibition of extracellular calcium ions influx, which is independent of potassium and voltage-dependent calcium channels (VDCCs). Except for VDCCs, a host of channels located at vascular smooth muscle plasma membranes are involved in mediating the influx of extracellular calcium, which includes non-selective cation channels, receptor operated calcium channels (ROCCs), store-operated calcium channels (SOCCs), etc.; one of which may be the target channel of Scu (Pan et al., 2008). Scu and its aglycone also inhibit Ca\(^{2+}\) overload and improve Ca\(^{2+}\)-ATPase activity through modulating the imbalance of excitatory amino acids, which cause excitotoxicity versus inhibitory amino acids. A study by Tang et al. (2015) demonstrates that a pre-treated administration of Scu and scutellarein attenuates the increases in intracellular aspartic, glutamate and glycine metabolic pathways, which are important in ischemia.
In vitro Scu exerted protective effects on the endothelium by inducing the expression of chaperonin proteins, protection of the p27BBP protein (EIF6) and maintaining the translation of factors important in cell survival (regulation of the translation of factors upstream of Bcl2/Bax). Proteomic analysis of protein expression profiles demonstrated that the p27BBP protein (EIF6), heat shock 60 kDa protein (HSPD1) and chaperonin containing the TCP1 subunit 6A isoform (CCT6A) could play important roles in the protective effects of Scu against ischemia and hypoxia injury. Pre-treatment of Scu at doses of 1, 5, and 10μM ameliorated the hypoxia-induced decrease in the level of the mentioned proteins in human cardiac microvascular endothelial cells (Shi et al., 2015a).

It is important to stimulate angiogenesis in the treatment of ischemia-associated tissue damage. In vitro experiments on human umbilical vein endothelial cells showed that Scu at 10μM induces endothelial cell proliferation, migration, invasion and capillary-like tube formation. Scu in a concentration-dependant manner induces MMP-2 activation and mRNA expression. The results suggest that it can be useful in angiogenic therapy (Gao et al., 2010).

Long-term administration of Scu at the doses 3, 10 and 30 mg/kg/day i.p. to rats with chronic myocardial infarction can improve the impaired cardiac function and protect against fibrosis and heart failure. It was found that Scu inhibits the proliferation and collagen production of cardiac fibroblasts. The mechanism of this action is connected to the inhibition of the expression of pro-fibrotic cytokine TGFβ1, and inhibition of p38 MAPK and ERK1/2 phosphorylation (Pan et al., 2011). In an experiment on human dermal fibroblasts it was demonstrated that Scu at 20μM and 30μM stimulates the expression of NF-κB, which is responsible for the inhibition of the collagen gene expression and in consequence the inhibition of collagen biosynthesis (Karna et al., 2011).

Neurodegenerative Diseases

Alzheimer’s disease (AD) is one of the most common devastating diseases of the central nervous system (CNS) with no curative treatments that are currently available. It is characterized by neuropathologic changes including increased plaques of toxic amyloid-β (Aβ) peptides in the extracellular space and neurofibrillary tangles consisting of hyperphosphorylated Tau protein. Inflammation and oxidative stress are other factors which have been associated with the pathogenesis of AD. The illness manifests through the loss of memory and cognitive functions (Pimplikar et al., 2010). Numerous pharmacological in vivo and in vitro trials exhibited the neuroprotective and neuromodulatory effects of Scu.

In AD patients, Aβ and the overexpressed levels of monoamine oxidases MAO A and B promote the production of reactive oxygen species (ROS) (Kennedy et al., 2003; Massaad et al., 2009). Pre-treatment of cortical synaptosomes with Scu (25–100 μmol/L) exerted in a dose dependent manner had a protective effect against oxidative neuronal damage induced by free radicals (Liu et al., 2003). As shown by Guo et al. (2013), the activity of MAO in the rat’s brain was reduced after oral administration of 10 mg of Scu over a period of 20 days.

The study found a significant reduction at cellular levels of the superoxide dismutase (SOD) in AD (Fukai and Ushio-Fukai, 2011). In animal cerebral ischemia model, Scu
administration restored the enzyme value to basic level (Tang et al., 2014, 2015). The results showed that an increase of SOD expression may prevent Aβ neuronal deposition and spatial and associative memory deficits (Guo et al., 2011, 2013).

Acute inflammation of the brain has been associated with AD pathogenesis. The excessive generation and secretion of pro-inflammatory components such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) induce many reactions in a vicious circle. These mediators may contribute to the accumulation of plaques of Aβ and Tau protein phosphorylation (Lee et al., 2009).

Overexpression of IL-1, IL-6 and TNF-α has returned to nearly regular values in the cerebral cortices of rats after a 20-day treatment with 10 mg of Scu/day through intragastric administration (Guo et al., 2013). An in vitro experiment on BV-2 mouse microglial and rat primary microglial cell line showed that Scu at a dose of 50 μM significantly inhibits the LPS-induced NO, TNF-α and IL-1β production, suppresses LPS-induced mRNA expressions of iNOS, TNF-α and IL-1β, and inhibits LPS-induced NFκB activation (Wang et al., 2011).

The main method of AD treatment (approved by the FDA) is to increase cholinergic activity through the use of selective cholinesterase or/and acetylcholine precursors. Studies have suggested that Aβ has a toxic effect on the nicotinic acetylcholine receptor (nAChR). The decrease in α3, α4 and α7 nAChR subunit proteins play a valid role in AD pathogenesis (Guan et al., 2000). In experiments on animals it was shown that Scu administration (10 mg intragastrically per day for 20 consecutive days) resulted in an up-regulation by 30% in α7 and 24% in α4 nAChR subunit proteins. No significant differences between the Scu treatment group and the positive control group (piracetam) were observed. Scu and piracetam at the same dose had similar effects. However, Scu was more effective in the improvement of cognitive deficits and enhanced the activity of ChE than piracetam (Guo et al., 2011, 2013). Moreover, the increased activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) around Aβ plaques could be restored to normal quality using Scu treatment (Guo et al., 2011).

To improve the properties of Scu, a series of carbamate derivatives were received as potential multifunctional agents designed and synthesized especially for the treatment of AD. The preliminary results indicated that most of the synthetic derivatives exhibited strong anti-oxidant potency, different AChE and BuChE inhibitory activities, selective chelation of metal ions and neuroprotective effects. Moreover, the new derivatives have been characterized by higher bioavailability after oral administration and a higher possibility of crossing the blood–brain barrier than Scu. The compounds can reach the biological targets located in the CNS. Among all of the derivatives, 4-(5-hydroksy-6,7-dimethoxy-4-oxo-4H-chromen-2-yl)phenyl N,N-dietycarbamate might have the greatest significance as a potential multifunctional agent in AD treatment. The compound exhibited the selective ability to chelate metal ions such as Cu2+, Al3+, Fe2+, Zn2+ and inhibited activities of AChE and BuChE (selectivity index for AChE = 39.7). The derivative has shown higher selectivity and better inhibition against AChE than rivastigmine which was used as the positive control (IC50 = 0.57 μM and 5.62 μM, respectively). The highest anti-oxidant
activity was also observed. Additionally, the derivative had high blood–brain barrier permeation ($P_e = 8.42 \times 10^{-6}$) (Sang et al., 2015).

Similar properties could be seen for a series of scutellarein-O-alkylamine derivatives. One of them, 5-hydroxy-4'-(3-((ethyl)(2-methoxybenzyl)amino)butoxy)-6,7-dimethoxyflavone, exhibited significant metal chelating properties, moderate AChE inhibitory, antioxidative activity, and excellent inhibitory effects on Aβ deposition (Sang et al., 2015).

Randomized clinical trials have shown the potential role of the estrogen replacement therapy (ERT) in AD prevention. Estrogens, especially 17β-estradiol, improve cognitive impairments in AD patients. However, ERT causes severe adverse effects, e.g. increases the risk of various forms of cancer. Therefore, the application of estrogen against AD is limited (Zhu et al., 2009; Dey et al., 2012). A potential candidate to substitute ERT in AD treatment might be Scu. It has shown estrogenic properties without stimulating cancer cell growth. Scu in a dose of 23 μg/mL exerted the same effects as 17β-estradiol in a dose of 2.7 ng/mL. The ERα activation was through inducing the phosphorylation of intracellular estrogen receptor α at the S1 18 position (Zhu et al., 2009).

Scu can also be utilized in other CNS diseases. In a mouse model of multiple sclerosis Scu-treated group of animals (50 mg/kg/day) showed a significant improvement of motor function and a decrease in demyelination. An in vitro study demonstrated that this effect is associated with the protective effect of Scu on neural stem cells (Wang et al., 2016a).

**Antitumor Activity of Scu**

*In vitro* and *in vivo* studies indicate potential use of Scu and scutellarein for the prevention and treatment of various human cancers. Xu and Zhang (2013) report that Scu is able to inhibit the proliferation and to induce the apoptosis of the human hepatocellular carcinoma cell line (HepG2) via the STAT3 signal pathway. The compound significantly decreased levels of anti-apoptotic proteins such as Bcl-xL and Mcl-1, which are the direct targets of the downstream of the STAT3 oncogenic pathway.

Scutellarein is an efficient inhibitor of activation and nuclear translocation of the NF-κB transcription factor, for which aberrant activation is associated with various aspects of oncogenesis, such as cancerous cell survival and proliferation, apoptosis prevention and increase in the metastatic potential. The down-regulation of the NF-κB expression contributes also to a decrease in the expression of the matrix metalloproteinases, specifically MMP-2, -9 and -14, which play crucial role in tumor metastasis (Shi et al., 2015b). The reduction in MMPs levels may link to previously described results for flavone apigenin which down-regulated STAT3 target genes MMP-2 and MMP-9. Therefore, the inactivation of STAT3 signaling pathway could also impair the cancer cells’ ability of migration and invasion through decreasing the MMP-2, -9 expression and activity (Cao et al., 2016). The MMP-2 and MMP-9 expression levels were found to be lower in Scu treated human tongue squamous carcinoma cells (SAS) (Li et al., 2013a,b). Moreover, it was revealed that low-intensity ultrasound may lead to increased cell sensitivity to Scu through increasing the cell membrane permeability and intracellular drug accumulation. The combination treatment using Scu and low-intensity ultrasound allows for the use of a much lower
concentration of this compound to achieve therapeutic effect (Li et al., 2013b). It was observed that Scu reduces also the level of integrin α_v β_6, without changing the E-cadherin mRNA expression, and increases the E-cadherin protein level which are related to changes in α_v β_6 integrin mRNA (Li et al., 2010, 2013a).

Another mechanism of Scu antitumor activity is the induction of the cell cycle arrest at G_0/G_1 transition. Scu inhibited the proliferation of B-lymphoma Namalwa cells at a low concentration (< 10 μM) through the down-regulation of cyclin D1 and CDK4 expression. At a high concentration (above 15 μM) Scu induced apoptosis, which is associated with the activation of caspases and mitochondrial pathways. The molecular mechanism of Scu action is unknown. Probably the compound enters the cell plasma membrane and binds to its unknown receptors in cytoplasm or the nucleus (Feng et al., 2012).

Scu exhibited antiproliferative action on human prostate PC3 cancer cells in a dose- and time-dependent manner. Cell proliferation was suppressed by promoting G2/M phase arrest and apoptosis induction. It was also determined that Scu in dose-dependent manner enhanced the toxicity of cisplatin against PC3 cells (Gao et al., 2017).

It was stated that Scu is a selective inhibitor of the proteasome catalytic subunits. The compound inhibited the chymotrypsin-like catalytic activity of this protein complex, but did not inhibit the trypsin-like and peptidyl-glutamyl peptide hydrolysing activities. The antiproteasome function prevents degradation of pro-apoptotic proteins and contributes to their accumulation. Those changes cause the induction of apoptotic cancer cell death (Wu et al., 2013). The inhibition of the proteasome by flavonoids and the potency of action are structure-dependent. Scutellarein, in contrast to its glycoside, does not inhibit the activities of the proteasome nor cause the accumulation of ubiquitous proteins, nor affects the proteasome target proteins. Nakamura et al. (2015) suggest that flavones with the hydroxyl groups at position 5, 6 and 7 of the A ring lack an antiproteasome function. However, the compounds with β-glycosylation of the C-7-OH group in the A ring, such as Scu, exhibit an antiproteasome action.

Recent examinations showed that Scu inhibits α', catalytic subunit of human protein kinase CK2 which is involved in enhancing sensitivity of cancer cells to programmed cell death (Baier et al., 2017).

The studies on many different human cancer cell lines confirmed that tumorigenesis correlates with an increase in the pyruvate kinase isoform M2 (PKM2) level. The isoform switch from PKM1 to PKM2 is necessary for cancer metabolism and tumor growth (Wu and Le, 2013). Earlier research has shown that PKM2 inhibitors may be used as a chemotherapeutic drug. Moreover, several studies have reported that PKM2 activators also suppress tumor growth in vitro (Gupta and Bamezai, 2010). There are not many studies on the effect of natural compounds on human PKM2 activity. Until now, only several flavonoids have been examined. Among them, Scu demonstrated the most potent PKM2 activation at low micromolar levels. The obtained IC_{50} value of Scu was 1.46 μM. The results suggest that the presence of the 4'-hydroxyl group in the Scu molecule determines strong activation of PKM2 by this compound (Aslan and Adem, 2015).

Additionally, Scu acts as a chemosensitizing agent. Chan et al. (2009) demonstrated that the compound sensitizes resveratrol and 5-fluorouracil evoked apoptosis of human
colon cancer cells through the enhancement of the p53-regulated caspase-6 activation. However, it should be noted that Scu itself does not trigger the apoptosis or caspase-6 activation at the tested concentration of 100 μM, and also it is unable to enhance the drug-evoked apoptosis in p53 knockout HCT116 cells.

**Antimicrobial Activity**

Preliminary *in vitro* studies demonstrate the anti-*Helicobacter pylori* activity of Scu, which is associated with its inhibitory effect on urease (HPU) (Yu et al., 2015). HPU is one of the most important virulence factors of *H. pylori*. It is essential for bacterial colonization of human gastric mucosa and is involved in the pathogenesis of gastric and peptic ulcers (Cellini and Donelli, 2000; Yu et al., 2015). Moreover, urease acts as a pro-inflammatory agent through mononuclear phagocytes’ activation and cytokines’ production (IL-1β, IL-6, IL-8 and TNF-α) (Harris et al., 1996). Scu effectively inhibits the enzyme catalytic activity in a concentration-dependent and time-independent manner. The tested flavone at a concentration of 1 mM was a more potent HPU inhibitor than the acetohydroxamic acid (AHA) which is a well-known HPU inhibitor. The IC$_{50}$ values (a concentration of the inhibitor which causes a 50% inhibition of the original enzyme activity) were 0.47 mM and 0.14 mM, respectively. Pharmacokinetic analysis suggests a non-competitive and reversible inhibition against HPU (Yu et al., 2015). The urease inhibitory effect of Scu depends on its chemical structure. The capacity to act as an HPU inhibitor is related to the amount and position of the hydroxyl groups in flavone skeleton. The hydroxyl group in the para position of the B ring improves the HPU inhibitory effect of the flavonoid most effectively (Yu et al., 2015). The most likely mechanism of HPU inactivation is the blocking of the sulfhydryl groups of the enzyme. The molecular docking analysis shows that amino acid residues such as Cys321, Asn168, Met366 and His221, which are located on the HPU mobile flap, are involved in the binding of Scu through hydrogen bonding. Among them Cys321 is the key residue responsible for the HPU catalytic activity. All interactions between the amino acid residues and Scu contribute to the reduction of the flexibility of the enzyme flap and therefore, its enzymatic activity (Yu et al., 2015).

The antibacterial activity of Scu was also determined against *Streptococcus pyogenes* and *Escherichia coli* with a minimum inhibitory concentration (MIC) value of 0.5 mg/mL, and antifungal activity against *Candida albicans* (MIC > 0.5 mg/mL) and *C. glabrata* (MIC 0.25 mg/mL) (Mamadalieva et al., 2011).

**Antiviral Activity against HIV and SARS**

Scu exhibits antiviral activity against several types of the human immunodeficiency virus (HIV-1). These include the laboratory-derived virus (HIV-1$_{IIIb}$), drug-resistant virus (HIV-1$_{24v}$) and low-passage clinically isolated virus (HIV-1$_{KMO18}$). The effective concentration, which inhibits 50% of viral production (EC$_{50}$), was 175 μM, 253 μM and 136 μM, respectively (Zhang et al., 2005). There is evidence that Scu possesses more than one mode of anti-HIV-1 action. The main possible mechanism of Scu action is the inhibition of the
retrovirus’ replication through decreasing the activity of the reverse transcriptase (HIV-1 RT). Moreover, Scu can interfere with various other processes of the retrovirus’ life cycle such as viral attachment and entry, cell fusion and cell–cell infection (Zhang et al., 2005). Scutellarein, apart from RT (Spedding et al., 1989), inhibited the HIV-1 protease (Ko et al., 2009). *In vitro* studies have shown that this compound demonstrates a similar or higher level of inhibition against HIV-1 RT ($IC_{50} = 53.1 \mu g/mL$) and protease ($IC_{50} = 71.7 \mu g/mL$) than even the well-known inhibitors such as the aurintricarboxylic acid ($IC_{50} > 100 \mu g/mL$) and pepstatin A ($IC_{50} = 51.0 \mu g/mL$) (Ko et al., 2009).

Scutellarein could be considered to be an effective anti-SARS (severe acute respiratory syndrome) virus agent. The compound strongly inhibits the ATPase activity of the SARS coronavirus helicase protein (nsP13), but not the dsDNA-unwinding activity. This enzyme is a critical component for virus genome replication. The $IC_{50}$ value was determined at 0.86 $\mu M$ (Keum and Jeong, 2012). Modeling analysis shows that scutellarein could fit in and directly interact with the ATP/ADP binding pocket of the SARS-CoV helicase protein, thereby excluding a direct binding of ATP/ADP. Scutellarein does not suppress the HCV viral helicase protein, which points to the structural selectivity of the compound. It is therefore important to examine which AA residues of the SARS-CoV helicase protein directly bind with this compound (Yu et al., 2012).

**Metabolic and Related Diseases**

It is possible that Scu can reduce the risk of atherosclerosis and coronary heart disease. After oral administration to rats (at doses of 30 mg/kg/day and 100 mg/kg/day), the flavonoid markedly reduced the total serum cholesterol and increased the serum HDL/LDL ratio. The study demonstrated that Scu improves the serum lipid profile and the atherogenic index similarly to simvastatin, which is a hypocholesterolemic and hypolipidemic drug commonly used in antiatherosclerotic treatment. Scu also enhances acetylcholine-induced nitrate/nitrite production, increases the eNOS gene expression and improves the acetylcholine-induced endothelium-dependent vasorelaxation (Li et al., 2009).

It was stated that Scu inhibited the enhanced activity of protein kinase C (PKC) isoforms (PKC $\beta I$, $\beta II$ and $\delta$) and their translocation from the cytosolic to the membrane fraction (Su et al., 2012). Persistent and excessive activation of several protein kinase C isoforms, caused primarily by high glucose levels, leads to the development of diabetic vasculopathy (Ishii et al., 1998). The flavonoid suppressed hyperglycemia-induced vascular inflammation in the human endothelial cells. This effect was related the decreasing of inflammatory factor levels, such as intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein 1 (MCP-1), and reduction of NF-$\kappa B$ nuclear translocation (Luo et al., 2008).

Scu exhibited an antiangiogenic effect on human retinal endothelial cells. The compound inhibited the ROS production, high glucose- and hypoxia-induced cell proliferation and migration. Through the inhibition of vascular endothelial growth factor (VEGF), it induced regression of neovascular retinopathy. The reduction of VEGF was due to increased ubiquitination and degradation of HIF-1$\alpha$ by Scu (Wang et al., 2014).
The treatment of diabetic rats with 100 mg/kg/day for two months of Scu resulted in decrease of hyperglycemia-induced ROS production, apoptosis and microcirculation impairment in testes. Data suggest that this compound can be a potential drug for prevention and treatment of diabetes-associated reproductive disorder (Long et al., 2015).

Other Activities of Scu

Among other activities of Scu is the protective action against immune-mediated liver injury. A dose of 100 mg/kg of the compound administered intraperitoneally to the mice decreased the level of hepatic enzymes (ALT and AST) elevated by concanavalin A and pro-inflammatory cytokine TNF-α in serum and TNF-α and iNOS mRNA expressions in the liver (Tan et al., 2007).

Another interesting finding is that Scu has the ability to inhibit [3H]-LSD binding to the serotonin receptor (5-HT7) with an IC50 value of 63.4 μM, which could be useful in the therapy of sleep disorders or depression (Gafner et al., 2003).

Pre-treatment of the human bronchial epithelial 16 (HBE16) cells with Scu inhibited the human neutrophil elastase (HNE)-induced MUC5AC expression, both at the mRNA and protein levels. The modulation of the MUC5AC expression through Scu appears to occur due to the inhibition of PKC and ERK1/2 signaling and is independent of STAT6 (Jiang et al., 2011b). The overexpression of the MUC5AC causes mucus hypersecretion and progression of the inflammatory process in the airway lumen. In vivo studies showed that Scu inhalations at the doses 50 mg/kg and 100 mg/kg considerably inhibited the MUC5AC expression and consequently, mucus production. The results suggest that it can be potentially used in the treatment of chronic inflammatory lung diseases (Jiang et al., 2011a).

The active concentrations of Scu in individual experiments were presented in Table 1. Various properties of Scu and signaling pathways are gathered together in Fig. 2.

Bioavailability and Pharmacokinetic Parameters of Scu

Experiments on rats showed that after oral administration, Scu has low bioavailability reaching 8% (Gao et al., 2011). The plasma concentration-time curve exhibits a double peak, first within an hour and second, higher, after a few hours. This suggests that the compound is absorbed in the stomach or the upper intestinal lumen and then after transformation by bacterial enzymes into the aglycone, it is absorbed in the colon. Gender-related differences in pharmacokinetic parameters could be observed, which could probably be connected with the activity of colon microflora (Xing et al., 2011). In the study on healthy human subjects after oral administration, it was found that the compound was biotransformed in liver. Most of the detected metabolites were formed through phase II conjugation. Four metabolites were found in urine samples. The major metabolite detected in plasma samples, apart from the parent compound, was scutellarein 6-O-glucuronide, which proves that the compound was first hydrolyzed by gastrointestinal microflora. The peak plasma concentration (Cmax) of the metabolite was estimated between 128.1 ng/mL.
and 38.11 ng/mL which was reached between 5 h and 11 h ($T_{\text{max}}$), while the elimination half-life ($T_{1/2}$) occurred between 2.22 h and 4.05 h. Its concentration in plasma was ~30-fold higher than Scu (< 5.0 ng/mL) (Chen et al., 2006; Gao et al., 2012). It was observed that the metabolites of Scu were eliminated not only with urine but also with bile (Gao et al., 2011).

Using hollow fiber cell fishing (HFCF) with HPLC/UV detection coupled with hollow fiber liquid-phase microextraction (HF-LPME) with HPLC/UV, Feng et al. (2017) investigated tissue distribution and pharmacokinetics of Scu after intraperitoneal injection of

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<tr>
<td>Relaxation of aortic rings</td>
<td><em>in vitro</em></td>
<td>$EC_{50}$ 1200 μM</td>
<td>Yang et al. (2009)</td>
</tr>
<tr>
<td>Anti-ischemic in heart ischemia</td>
<td><em>(rats)</em></td>
<td>15 mg/kg and 50 mg/kg</td>
<td>Lin et al. (2007)</td>
</tr>
<tr>
<td>Anticancer: apoptosis of HepG2</td>
<td><em>in vitro</em></td>
<td>30 μM and 100 μM</td>
<td>Xu and Zhang (2013)</td>
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<td>hepatocarcinoma cell</td>
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<tr>
<td>inhibition of Burkitt lymphoma</td>
<td><em>in vitro</em></td>
<td>$IC_{50}$ 16.65 μM</td>
<td>Feng et al. (2012)</td>
</tr>
<tr>
<td>Namalwa cell</td>
<td><em>(mice)</em></td>
<td>15 mg/kg/day</td>
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<td>inhibition of prostate cancer PC3 cell</td>
<td><em>in vitro</em></td>
<td>200–600 μM</td>
<td>Gao et al. (2017)</td>
</tr>
<tr>
<td>inhibition of HL60 leukemia cell</td>
<td><em>in vitro</em></td>
<td>$IC_{50}$ 13.58 μM</td>
<td>Wu et al. (2013)</td>
</tr>
<tr>
<td>inhibition of A549 lung cancer cell</td>
<td><em>in vitro</em></td>
<td>$IC_{50}$ 15.1 μM</td>
<td>Wu et al. (2013)</td>
</tr>
<tr>
<td>inhibition of oral squamousal cancer cell</td>
<td><em>in vitro</em></td>
<td>75 nM</td>
<td>Li et al. (2010)</td>
</tr>
<tr>
<td>Anti-atherosclerotic</td>
<td><em>(rats)</em></td>
<td>30 mg/kg/day and 100 mg/kg/day</td>
<td>Li et al. (2009)</td>
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<td>Prevention of hyperglycemia-induced vascular complications</td>
<td><em>in vitro</em></td>
<td>0.1 μM and 1 μM</td>
<td>Luo et al. (2008)</td>
</tr>
<tr>
<td></td>
<td><em>(mice)</em></td>
<td>50 mg/kg/day (22 days)</td>
<td>Luo et al. (2008)</td>
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<tr>
<td></td>
<td><em>(rats)</em></td>
<td>100 mg/kg/day (10 weeks)</td>
<td>Su et al. (2012)</td>
</tr>
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</table>
Figure 2. Therapeutic potential of Scu.
breviscapine in vivo on rats. It was observed that Scu can be rapidly absorbed into the bloodstream and can metabolize into scutellarein. After 0.5 h, the concentration of both compounds in plasma was almost identical and both reached pathologically changed tissues (in this case cancerous liver and kidney cells) achieving the therapeutic concentration. In the kidney, they had similar concentrations, but in the liver the concentration of scutellarein was significantly higher than Scu.

The in vivo animal studies showed that the most promising route of delivery of Scu to the body is an inhalation. The absolute bioavailability of Scu after intratracheal administration was found to be over 77% (Liu et al., 2008).

Improvement of Bioavailability

Isolated from plant material, Scu has low water- and lipid-solubility, low absorption and bioavailability. A method to improve the absorption of Scu could be the synthesis of polyethylene glycol-Scu amide prodrugs using methyl polyethylene glycols (mPEGs) with different molecular weight as a carrier which remarkably increase the solubility in water and in lipids (Lu et al., 2010). Water solubility also increased Scu-cyclodextrin conjugates (Yang et al., 2013). In a recent study it was observed that surfactant Cremophor EL enhanced the transportation of Scu through biomembranes by MRP3 (multidrug resistance-associated protein) transporter. Additionally this substance inhibited the activity of efflux transporters MRP2 and BCRP (breast cancer resistance protein) (Xiao et al., 2016). To improve the bioavailability of the Scu nanoparticles based on vitamin B12-modified amphiphilic chitosan derivatives (Chit-DC-VB12) were also utilized (Wang et al., 2017).

Toxicity and Interactions of Scu

Scu is regarded as a safe compound. Acute and subacute toxicological studies carried out on rodents showed that this compound is non-toxic and the maximum tolerated dose is more than 10 g/kg. The dose commonly used in humans is 50 mg three times per day and is far less than the acute non-observed adverse effect level (Li et al., 2011).

It is important to notice that Scu in high doses can have an influence on drug metabolizing enzymes. It inhibits several isoforms of cytochroms P450 (IC₅₀ > 100 μM) which may cause interactions with synthetic drugs (Han et al., 2014). At the concentration 100 μM scutellarein in vitro, Scu inhibited the activity of UDP-glucuronosyltransferase (UGT) isoforms (UGT1A1, UGT1A6, UGT1A9, UGT2B7). Moreover the aglycone demonstrated a stronger effect than glycoside (Ma et al., 2014).

Modern Methods of the Isolation of Scu

It is rather difficult to obtain Scu from plant material in its pure state. There exist various elaborate methods for its isolation. One of them is preparative high-speed counter-current chromatography (HSCCC) (Gao et al., 2006). This method allows us to obtain the compound with high purity although it is expensive. A method adopted for the isolation of Scu
is the microwave-assisted extraction. It is better than the traditional heat-reflux method because there is a reduction in extraction time and high recovery (Gao et al., 2007b). An efficient method to concentrate Scu in an extract is the adsorption–desorption process on macroporous resins. The best result was obtained with HPD-800 resin which has a large surface and moderate polarity and a high percentage of adsorption and desorption. This method can be utilized to obtain a large amount of Scu from crude extracts for commercial use (Gao et al., 2007a).

Conclusions

Scu has been shown to exert various beneficial biochemical effects, which can be useful most of all in the curing of cardiovascular diseases. Preliminary results showed that Scu could be an important antineurodegenerative, anticancer or anti- Helicobacter drug, and could be used as the leading compound in designing new multi-targeted therapeutic agents. Invented derivatives of Scu possess better pharmacokinetic properties than the parent compound and seem to be promising drugs. In in vitro experiments, they showed the same or even higher activity than synthetic drugs.

Low solubility and absorption of Scu, resulting from its low bioavailability, can be a limitation, but nowadays these issues are easily solved if modern forms of drug are applied and its therapeutic abilities can be fully utilized.

Conceived on a wide scale, Scu examinations started from identifying it as the main component of the herb of E. breviscapus, which has been widely used in TCM in the treatment of cerebral and heart vascular diseases. Traditional use of the plant in these complaints was fully confirmed on the basis of modern pharmacological examinations and the results suggest that Scu is largely responsible for this action.

Possessing many bioactivities, Scu has the potential to be developed as a new multifunctional drug that will make contributions to human health. However, more scientific evidence based on clinical studies are seriously needed.

References


