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# Clinical benefits and pharmacology of scutellarin: A comprehensive review

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ABSTRACT

Stroke and myocardial infarction are among the most common causes of mortality and disability in the world. The ischemic injury underlying these illnesses is complex, involving intricate interplays among many biological functions including energy metabolism, vascular regulation, hemodynamics, oxidative stress, inflammation, platelet activation, and tissue repair that take place in a context- and time-dependent manner. The current drug therapy of choice is to timely resupply the blood to the ischemic tissue; but reperfusion may introduce additional harm to the tissue through a process known as ischemia/reperfusion injury. As such, new drugs that would complement reperfusion by providing neural and cardiovascular protection and by targeting multiple abnormalities in ischemia are receiving increased attention. Scutellarin is an herbal flavonoid glucuronide with multiple pharmacological activities. Owing to its multiple beneficial effects, such as anti-oxidant, anti-inflammation, vascular relaxation, anti-platelet, anti-coagulation, and myocardial protection, scutellarin has been used clinically to treat stroke, myocardial infarction, and diabetic complications. Over the past three decades, clinical and pharmacological studies have accumulated a body of evidence that not only demonstrated these therapeutic effects, but also provided significant insights into the pharmacokinetic behavior, therapeutic profile, and mode of action of scutellarin in humans and animal models. Medicinal modification and new drug delivery methods have led to the development of new derivatives and formulations of scutellarin with improved bioavailability, efficacy, and safety. Here we review the current literature on scutellarin to provide a comprehensive understanding of the pharmacological activity, mechanism of action, toxicity, and therapeutic potential of scutellarin for the treatment of ischemia, diabetic complications, and other chronic diseases.



**Contents**



*Abbreviations:* Aβ, β-amyloid peptide; ACE, angiotensin-converting enzyme; AD, Alzheimer's disease; ADE, adverse drug effect; ADR, adverse drug reaction; AIF, apoptosis-inducing factor; Akt, protein kinase B or PKB; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; Ang II, angiotensin II; AT1R, Ang II type 1 receptor; $AUC_{0-\infty}$, area under the curve from time 0 to the last measurable concentration; BBB, blood-brain barrier; BCCAO, bilateral common carotid artery occlusion; BCRP, breast cancer resistance protein; CI, confidence interval; $C_{max}$, maximal plasma concentration; CNS, central nervous system; CYP, cytochrome P-450; ECG, electrocardiogram; eNOS, endothelial nitric oxide synthase; Erk, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; HCC, hepatocellular carcinoma; HDL, high density lipoprotein; i.g, intragastric administration; iNOS, inducible nitric oxide synthase; i.p., intraperitoneal injection; i.v., intravenous administration; IL, interleukin; IOP, intraocular pressure; I/R, ischemia/reperfusion; LAD, left anterior descending; LDL, low density lipoprotein; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; miR-7, microRNA-7; MMP, matrix metalloproteinase; MNCV, motor nerve conduction velocity; MRP, multidrug resistance-associated protein; $MRT_{(0-t)}$, mean residence time; NAD, nicotinamide adenine dinucleotide; NF-κB, nuclear factor κ-light chain-enhancer of activated B cells; NO, nitric oxide; NSCLC, non-small cell lung cancer; NYHA, New York Heart Association; OATP, organic anion-transporting polypeptide; OR, odds ratio; PARP, poly (ADP-ribose) polymerase; PEG, polyethylene glycol; P-gp, P-glycoprotein, multidrug resistance protein or MDR; PKC, protein kinase C; PKG, protein kinase G; RNS, reactive nitrogen species; ROS, reactive oxygen species; S, scutellarein, 5,6,7,4′-tetrahydroxyflavone; S-7-G, scutellarin, scutellarein-7-*O*-glucuronide; S-6-G, isoscutellarin, scutellarein-6-*O*-glucuronide; S-6,7-diG, scutellarein-6,7-*O*-diglucuronide; SBP, systolic blood pressure; SMD, standardized mean difference; SNCV, sensory nerve conduction velocity; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription protein 3; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; TGF-β1, transforming growth factor β1; TNF-α, tumor necrosis factor-α; TTC, triphenyl tetrazolium chloride; UGT, uridine 5′-diphospho-glucuronyltransferase; VEGF, vascular endothelial growth factor; WMD, weighted mean difference.

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## 1. Introduction

Scutellarin is a flavonoid drug derived from the plant *Erigeron breviscapus* (Vant.) Hand.-Mazz., a Chinese herbal medicine with multiple pharmacological effects and clinical applications. The whole plant was used to treat paralysis caused by stroke and joint pain from rheumatism by the Yi minority people of Southwest China for generations (Wang, Yang, and Yang, 2012). The structure of scutellarin was determined to be a glucuronide conjugate of 5,6,7,4′-tetrahydroxyflavone (scutellarein, S, $C_{15}H_{10}O_6$) at the 7-*O* position (scutellarein-7-*O*-glucuronide, S-7-G, $C_{21}H_{18}O_{12}$) a century ago (Fig. 1a and b). However, the systematic study of scutellarin in modern medicine did not begin until the late 1970s, when China launched a large campaign to identify and modernize therapeutics from traditional Chinese medicine. This campaign has been credited with the discovery of a number of clinically successful drugs, best exemplified by the antimalarial drug, Artemisinin, derived from the herbal medicine *Artemisia annual L.* (Foundation, 2015). Scutellarin was identified as a major active ingredient of *Erigeron breviscapus* from these early studies (Medica, 1976; Wang et al., 2012). Breviscapine, the total flavonoid extract of *E. breviscapus* containing ≥90% Scutellarin and ≤10% apigenin-7-O-glucronide in content, was classified as a prescription drug. At present, more than ten million patients use breviscapine and related drugs each year in China (Liu et al., 2018).

The research on scutellarin in the past three decades has led to the accumulation of a large body of evidence that establishes the effectiveness of the drug in treating cerebrovascular and cardiovascular diseases, in particular, ischemic stroke and coronary heart disease (Chinese Pharmacopoeia Commission, 2015; Gao et al., 2017; Yang, Cheng, Xie, Yang, and Zhuang, 2012; Yang, Li, Xie, Zhuang, and Yang, 2013). Clinical and laboratory studies have also implicated scutellarin in treating several other chronic illnesses, such as diabetic complications (Li, Wu, and Wang, 2009; Liu et al., 2016; Wu, Zhong, and Sun, 2002; Zheng, Ou, Shen, Zhou, and Wang, 2015). Moreover, these studies revealed significant insights into the pharmacologic aspects of the drugs, including pharmacokinetic behavior, mode of action, drug target, and adverse reaction, in both humans and animal models (Gao et al., 2012; Gao, Chen, and Zhong, 2011; Li, Lin, Xie, Zhang, and Guo, 2015; Li, Wang, Li, Bai, and Xue, 2011; Lin et al., 2007; Yuan, Fang, Wu, and Ling, 2016; Yuan, Zha, Rangarajan, Ling, and Wu, 2014). Additionally, advances in the

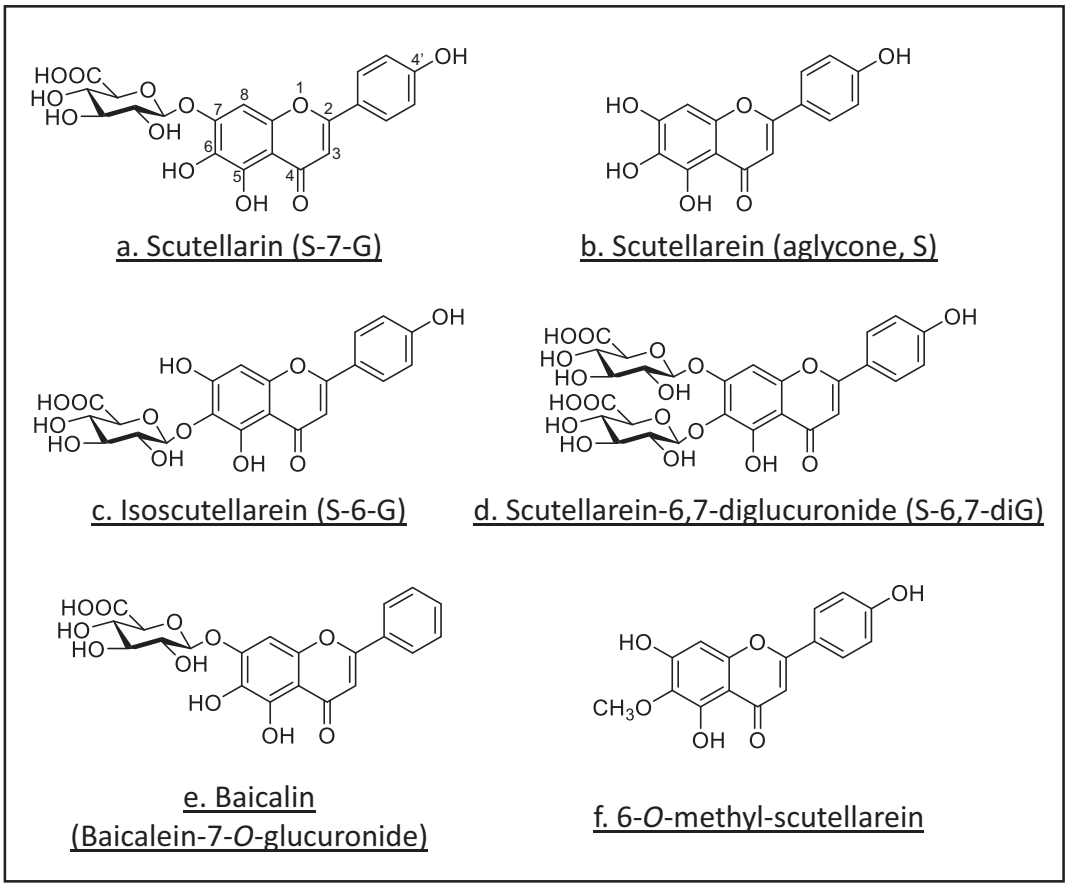


**Fig. 1.** Structures of scutellarin and related compounds. The structures of (a) scutellarin (scutellarein-7-*O*-glucuronide, S-7-G, $C_{21}H_{18}O_{12}$); (b) scutellarein (aglycone, 5,6,7,4′-tetrahydroxyflavone, S, $C_{15}H_{10}O_6$); (c) isoscutellarin (scutellarein-6-*O*-glucuronide, S-6-G); (d) scutellarein-6,7-*O*-diglucuronide (S-6,7-diG); and (e) baicalin (baicalein-7-*O*-glucuronide, $C_{21}H_{18}O_{11}$), an analogue of scutellarin lacking the 4′-hydroxyl group.

formulation, drug delivery, and structural modification of scutellarin in recent years opened new opportunities for improvement of the bioavailability, therapeutic profile, efficacy, and safety of scutellarin (Lu et al., 2010, 2012; Wang et al., 2017). In this article, we review the literature on scutellarin in order to provide a comprehensive understanding of the therapeutic effect, pharmacokinetics, and pharmacological activity of the drug.

## 2. Therapeutic effect and clinical benefit

*Erigeron breviscapus* (Vant.) Hand.-Mazz., also known as *Herba Erigerontis*, *Lamp Chrysanthemum*, and fleabane, or dengzhanxixin and dengzhanhua in Chinese, is found in many mountainous areas of Southwest China. In addition to treating paralysis and rheumatism by the Yi people, the herbal medicine was used to heal headache, toothache, gastritis, and fever by the Yi, Zhuang, and Tibetan minority groups for hundreds of years (Medica, 1976; Wang et al., 2012).

The therapeutic development of *E. breviscapus* includes both its pure active ingredients and extract preparations that are better defined, more stable, and easier to use than the herbal medicine itself. Breviscapine is the clinically most widely used crude extract from the herb. It consists mainly of flavonoids, with scutellarin as a major component for both its chemical composition (≥90%) and pharmacologic activity (Tian, Zhao, Gu, Cai, and Yu, 2014; Zhang, Zhang, Wang, Lin, and Shang, 1988). Therapeutic breviscapine preparations include injectables, capsules, and tablets. The concentrations of scutellarin in these preparations are set to be larger than 90.0% in oral preparations and larger than 98.0% in injectable preparations according to Pharmacopoeia of the People's Republic of China 2015 (Chinese Pharmacopoeia Commission, 2015). Variations in the composition and bioactivity among different preparations exist, which is in part caused by variations in the extraction and packaging methods used by different manufacturers. For instance, the dengzhanxixin injection, a clinically used preparation, contains several isomers of caffeic acid esters ($C_{25}H_{24}O_{12}$) in addition to flavonoids. These extract preparations, along with scutellarin and its derivatives, have been used in drug therapy to treat a range of illnesses, often in conjunction with Western medicine and other Chinese medicine drugs. Accumulated data have demonstrated the effectiveness and benefits of breviscapine and scutellarin in treating cerebrovascular disease, cardiovascular disease, and diabetic complication in clinics and in experimental systems.

In the practice of traditional Chinese medicine, stroke, heart attack, and diabetic complications are believed to result from “qi deficiency” and/or “blood stasis”—terms used to describe disease conditions caused by deficiencies in body and organ functions and in circulation, respectively, in traditional medicine. Accordingly, these disease conditions are treated with “tonifying qi” and/or “blood circulation promoting” drugs to which *Erigeron breviscapus* belongs (Zhou, Guo, and Gao, 2015). In this connection, the clinically proven therapeutic effects of scutellarin and breviscapine preparations on cerebrovascular and cardiovascular diseases appear to be in a good agreement with the “qi deficiency/blood stasis” theory of traditional Chinese medicine.

It is imperative to point out that, because many extract preparations are essentially natural products with mixtures of known and unknown components, it is critical to adopt a high standard of quality control in both the manufacturing and clinical use of the herbal extracts, in order to minimize product variations and obtain consistent and quantitative therapeutic effects. For this purpose, the China Food and Drug Administration issues the Pharmacopoeia of the People's Republic of China to be used as a standard (Chinese Pharmacopoeia Commission, 2015). On the other hand, scutellarin in its pure form exhibits a low solubility, low bioavailability, and short half-life in biological systems, which limit its efficacy in some clinical applications (Chen, Cui, Duan, Ma, and Zhong, 2006; Gao et al., 2012; Liu and Xiong, 2009). For instance, the bioavailability of scutellarin from an oral administration in Beagle dogs is merely 0.40% ± 0.19% and its half-life 79.98 ± 44.50 min (Ge, Zhou, Zhi, Ma, and Chen, 2003). For these reasons, medicinal and formulation modification of scutellarin to improve its bioavailability and efficacy has become an important approach for new drug development from scutellarin. Nonetheless, pure scutellarin and its derivatives are commonly used for pharmacological and pharmacokinetic studies in animal and in vitro models, whereas a majority of the clinical therapeutic effects of scutellarin are observed with breviscapine preparations at the present time, as discussed in more detail below.

It is also worth noting that many published clinical studies on the therapeutic effects of scutellarin and breviscapine were preliminary and inconclusive, which is in part attributable to the lack of sufficient patient enrollment, quantitative endpoints, a randomized and double blinded design, appropriate controls, and statistical power. As a result, meta-analyses are often performed to combine randomized clinical trials with small sample sizes. Nevertheless, large scale, randomized and controlled, and prospective clinical trials remain much needed for the evaluation of the efficacy and safety of scutellarin and related pharmaceuticals.

### 2.1. Cerebrovascular disease

Stroke is a leading cause of mortality in both developed and developing countries with a lifetime risk of about 10% (Seshadri et al., 2006). Worldwide, there are 31 million stroke survivors and about 6 million deaths are due to cerebrovascular disease, representing the second most common cause of death and the sixth most common cause of disability (Ward, Toledano, Shaddick, Davies, and Elliott, 2015). A stroke occurs when blood supply to part of the brain is blocked causing ischemic tissue injury, or when a cerebral blood vessel bursts leading to intracranial hemorrhage. In either case, parts of the brain become damaged, resulting in lasting brain lesions, long-term disability, and death. As a result, cerebrovascular brain injury has attracted increasing attention in drug discovery. In the case of ischemic brain injury, which accounts for 88% of all stroke, the thrombolytic therapy designed to restore cerebral perfusion in a timely fashion is the conventional approach. However, this ischemia/reperfusion (I/R) approach may lead to increased inflammation, oxidative stress, heightened glial response, and excitotoxicity in the brain from blood reperfusion itself, exacerbating tissue damage. This notion provides a rationale for including neuroprotective agents in the treatment of stroke (Cuzzocrea, Riley, Caputi, and Salvemini, 2001; Green, 2008). In this regard, a number of traditional Chinese medicine-derived drugs, including breviscapine and scutellarin, have been utilized for treating cerebrovascular accidents and disorders, owing to their anti-inflammatory, anti-oxidative, and beneficial vascular and hemodynamic effects, in addition to their low toxicity, low cost, and ease of access (Ghosh et al., 2014; Gu, Chen, and Shen, 2014; Shang, Tian, Hou, and Xu, 2013; Wu et al., 2007; Wu, Fang, Karthikeyan, Yuan, and Ling, 2017; Wu, Zhang, Wang, and Chen, 2010; Yuan et al., 2016; Zhou et al., 2015).

In an early study of 100 patients with acute cerebral infarction, the clinical curative effect of the dengzhanhua injection, a breviscapine preparation, was found to be as high as 92% (Zhang, He, and Li, 2002). A significant improvement in the hemorheologic and blood lipid parameters, as well as neuronal function and infarct absorption, was observed in patients receiving the dengzhanhua treatment, compared with control. No apparent adverse drug effects were found from the treatment. A meta-analysis of clinical studies on all traditional Chinese patent medicine for ischemic stroke from 1992 to 2004 listed two dengzhanxixin preparations as among the drugs that produced marked improvement in neurologic deficits, which are considered as secondary outcomes of ischemic stroke, with no severe adverse events (Wu et al., 2007). However, the effect of the drug on the primary outcomes, i.e., death and dependency, was inconclusive, due to the lack of randomization, double blinded design, and placebo-control. A Cochrane review of the early randomized, quasi-randomized, and controlled clinical trials prior to 2008 on dengzhanhua preparations in patients with confirmed acute cerebral

infarction provided a similar conclusion, confirming improvement of neurologic functions by the drugs, but no conclusive benefits on the death rate or other primary outcomes, as well as concerns over the appropriateness of the methodology used in and the quality of the data obtained from these clinical trials (Cao, Liu, Wu, Zhong, and Liu, 2008).

To address concerns over study size and standardization, data from the hospital information system of twenty hospitals across China were collected to analyze the clinical safety and therapeutic effectiveness of the dengzhanxixin injection (Yang et al., 2012). A total of 21,498 patients were recruited with 78% in the ages between 45 and 80 years old and more males than females. In most cases, the dengzhanxixin injectable was given within 3 days after hospitalization at a dose between 30 and 40 mL each time for 8 to 12 days. Common concomitant medications were atorvastatin, probucol, and aspirin for cerebral infarction, and metoprolol, aspirin, and isosorbide dinitrate for coronary heart disease. A total of 2512 cases of cerebral infarction were separated into an observation group receiving the dengzhanxixin injection (1008 patients) and a control group without the dengzhanxixin treatment (1504 patients). By using generalized and boosted models to score weighted regression on age, gender, and a set of 72 variables, a statistically significant difference was found in the mortality rate between the two groups ($p < 0.001$), indicating that the dengzhanxixin treatment reduced the death rate in patients with cerebral infarction (Yang et al., 2013).

In traditional Chinese medicine practice, breviscapine is considered as a drug that promotes "blood circulation" and can be used together with drugs that tonifies "qi"—drugs that enhance body or organ functions—in addition to symptomatic treatment and conventional antistroke medicine, to treat cerebral ischemic injury clinically. This combined therapy has been shown to be safe and more efficacious and beneficial than using the drugs individually (Zhou et al., 2015). A study in 2011 evaluated the effect of a combined therapy with the dengzhanxixin injection and the shengmai capsule on stroke (Wei et al., 2011). In a multicenter, prospective, and centrally randomized study, 678 patients with ischemic stroke were divided into (a) the dengzhan group (343 patients) receiving the dengzhanxixin injection in the acute phase of stroke and the dengzhan shengmai capsule in the recovery phase, plus symptomatic treatment, and (b) the Western medicine group (335 patients) receiving Aspirin enteric-coated tablet and Western medicine health education, plus symptomatic treatment. The treatments were for 180 days with a follow-up to 360 days. The results revealed that the dengzhan group had improved primary outcomes: (a) a fatality rate of 1.17% for the dengzhan group, compared with 4.78% of the Western medicine group ($p < 0.05$), and with the general fatality rate of ischemic stroke of 5.9–6.6% from the literature; (b) a disability rate of 39.53% in the dengzhan group, compared with 40.13% of the Western medicine group; and (c) a relapse rate of 3.21%, compared with 3.59% of the Western medicine group ($p < 0.05$) and with the general relapse rate of 5.9% from the literature. The findings revealed that the dengzhanxixin injection, when combined with "tonifying qi" drugs, improves the primary outcomes in patients with ischemic stroke, which is on par with or moderately advantageous over that of conventional Western medicine treatments.

A recent randomized clinical trial tested a combination treatment for acute cerebral infarction with the dengzhanhua injection, the xiongqin injection, and the xuesaitong treatment, compared with xuesaitong alone. The study was carried out in 140 elderly patients with 70 cases for each of the two groups (Huang, He, and Lei, 2014). The findings revealed that the combination treatment had a significantly higher curative rate than the control, with improvements in the cerebral blood flow, plasma viscosity, platelet adhesion, neuronal functions, and life skills. These results support a synergistic and/or complementary effect of the dengzhanxixin preparation with other traditional Chinese medicines in treating cerebrovascular accidents.

### 2.2. Cardiovascular disease

Cardiovascular disease continues to represent the leading cause of death worldwide. In 2015, the disease resulted in 17.9 million deaths, accounting for 32.1% of all death (GBD 2015 Mortality and Causes of Death Collaborators, 2016). Coronary heart disease (ischemic heart disease), including various types of angina pectoris, myocardial infarction, and sudden cardiac death, is the most common type of cardiovascular disease (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). Coronary artery disease affected 110 million people and resulted in 8.9 million deaths, which was 15.9% of all deaths in 2015 globally (GBD 2015 Mortality and Causes of Death Collaborators, 2016). Breviscapine is widely used in China for the treatment of cardiovascular diseases, especially, coronary heart disease, because of its multiple beneficial effects on the cardiovascular system, including vasodilation, myocardial protection, anti-inflammation, anticoagulation, anti-thrombosis, anti-oxidative stress, and vascular endothelial and smooth muscle cell protection. Breviscapine is often used in conjunction with conventional Western medicine for the treatment of cardiovascular diseases, which demonstrates improved efficacy and benefits to the patients in comparison with conventional treatment alone.

#### 2.2.1. Coronary artery disease

A meta-analysis was conducted using 16 studies from 2001 to 2012 involving 1505 patients (Wang et al., 2015). The studies were randomized and controlled trials, with participants suffering from and being treated for angina pectoris. Breviscapine injection was used in the treatment group. The treatment lasted for a minimum of 14 days and maximum of 15 days. The improvement rate of angina pectoris symptoms, defined as at least 50% reduction in symptoms, was significantly higher for patients treated with breviscapine plus Western medicine, compared to patients treated with Western medicine alone (92% vs. 76%; odds ratio or OR: 3.77; 95% confidence interval or CI: 2.76–5.15; $p < 0.05$). The combined therapy was also better than the Western medicine monotherapy in achieving electrocardiogram (ECG) improvement (81% vs. 62%; OR: 2.77; 95% CI: 2.16–3.53; $p < 0.05$). The overall adverse events occurred less frequently for the combined therapy than for Western medicine alone, including headache, erubescence, itching skin, palpitations, and fatigue. The authors concluded that breviscapine plus Western medicine produced a superior therapeutic effect to that of Western medicine alone.

In a separate meta-analysis, 17 randomized and controlled trials were combined to analyze the treatment of unstable angina with the dengzhanxixin injection (Nie et al., 2012). The treatment group included 765 patients treated with the dengzhanxixin injection plus conventional treatments, while the control group consisted of 727 patients receiving conventional treatments only. A fixed effect model was used to carry out the meta-analysis. The treatment effects in the treatment group were significantly better than in the control group ($p < 0.00001$). The effect of the dengzhanxixin injection on ECG was analyzed by combining 16 studies with 784 patients in the treatment group and 740 patients in the control group. The treatment effects with the dengzhanxixin injection were significantly better than those in the control group for ECG improvement ($p < 0.00001$).

The influence of breviscapine injection on coronary heart disease outcomes was analyzed using the hospital information system from 20 hospitals across China (Lu et al., 2013; Shen, Yang, Xie, and Zeng, 2013). A total of 2325 cases of coronary heart disease were divided into an observation group receiving the dengzhanxixin injection plus conventional treatments (768 cases) and a control group receiving conventional treatments only (1557 cases). By using generalized and boosted models to score weighted regression on age, gender, and a set of 72 variables, three types of logistic regression analyses were applied to identify the cure rate and the mortality of coronary heart disease. A significant difference was found in the cure rate and the mortality rate

between the observation and control groups, indicating that the combined use of dengzhanxixin injection with conventional treatments improved the cure rate and reduced the mortality rate of patients with coronary heart disease compared with conventional treatments alone.

*2.2.2. Myocardial infarction*

Myocardial infarction is a heart attack due to blockage of blood flow in the coronary artery branches to a region of the heart, often caused by a thrombus on a ruptured atherosclerotic plague, to result in infarction of the myocardium. A randomized and controlled trial was conducted to analyze the effect of breviscapine plus conventional medicine, compared with conventional medicine alone, on patients with cardiac infarction after percutaneous coronary intervention. The proportion of less than or equal to New York Heart Association (NYHA) functional class II of heart failure in the test group was found to be higher than that in the control group (88.3% vs. 61.7%), whereas the incidence of cardiac adverse events, i.e., myocardial infarction, arrhythmia, and death, was lower in the test group than in the control group (6.7% vs. 21.7%) (Yang and Chen, 2013). The effect of breviscapine on exercise tolerance in patients with acute myocardial infarction after a successful thrombolytic treatment was analyzed in a randomized trial. A total of 98 patients were randomly assigned to a combination group (breviscapine plus conventional treatment) or a control group (conventional treatment alone) for 14 days. Treadmill exercise showed a significant prolongation of the time before exercise-induced ECG ST-segment depression (≥0.1 mV) and shortening of the duration of ST-segment depression in the combination group than in the control group on the 36th day (Wang, Huang, and Zhang, 2009).

The above studies indicate that addition of breviscapine to conventional treatments produces significantly better results for patients with coronary heart disease than conventional treatments alone, increasing the cure rate, reducing mortality, improving the symptoms and ECG in angina pectoris patients, and improving the recovery from myocardial infarction.

Breviscapine in conjunction with conventional medicine is also beneficial in treating other cardiovascular diseases, including hypertension, congestive heart failure, hyperlipidemia, arrhythmia, and pulmonary heart disease, in randomized and controlled clinical trials, though meta-analysis of the clinical trials and large-scale, randomized clinical trials are generally lacking for these clinical effects (Gao et al., 2017).

*2.2.3. Hypertension*

Hypertension is a long-term medical condition in which the arterial pressure is persistently elevated. Uncontrolled high blood pressure is a major risk factor for coronary artery disease, stroke, heart failure, vision loss, peripheral vascular disease, and chronic renal disease, accounting for 9.4 million deaths or 18% of all deaths in 2010 globally (Campbell et al., 2015). Traditional Chinese patent medicine has been widely used for treating essential hypertension, often together with anti-hypertensive drugs of Western medicine. In a randomized and controlled trial, 76 patients suffering from essential hypertension were treated with either amlodipine (an angioselective calcium channel blocker) and captopril (an angiotensin-converting enzyme or ACE inhibitor)/uropidil (an α1-adrenoceptor antagonist) (control group) or the above plus breviscapine (intravenous drip for two treatment courses, treatment group) (Wei and Tan, 2005). The combined therapy with breviscapine and anti-hypertensive drugs decreased blood pressure and improved renal function more effectively and significantly, reducing the urinary β2-microglobulin and the 24-h urinary protein excretion, than the anti-hypertensive drugs alone. In another clinical trial, an extract injection with scutellarin as a major ingredient at a daily dose of 40 mL was shown to produce similar anti-hypertensive effects to enalapril (an ACE inhibitor) at a daily dose of 20 mg in elderly patients with essential hypertension. Moreover, Erigeron reduced the urinary excretion of β2-microglobulin and N-acetyl-beta-d-glucosaminidase, markers of renal tubular damage (Wang, 2000).

*2.2.4. Congestive heart failure*

Congestive heart failure is a common, costly, and potentially fatal condition, affecting about 40 million people in 2015 globally (Disease et al., 2016). It occurs when the heart is unable to pump sufficiently to maintain blood flow to meet the body's needs, often as a result of coronary artery disease. In a randomized and controlled trial of 126 patients with chronic heart failure of stage NYHA II to III, breviscapine was given to patients at 50 mg per day together with conventional medicine, whereas the control group received conventional medicine alone (Tian, 2010). The combination treatment effectively reduced the left ventricular end-diastolic volume and improved the 6-minute walk test compared with the control. In another randomized controlled trial, breviscapine was given to patients suffering from heart failure of stage NYHA III to IV with a normal ejection fraction at 40 mg per day together with routine medicine (Zhang, 2014). The combination treatment decreased the B-type natriuretic peptide and the typical symptoms of heart failure more effectively than the routine treatment alone, but there was no difference in the left ventricular ejection fraction and the left ventricular end-diastolic volume between the two groups.

*2.2.5. Hyperlipidemia*

Hyperlipidemia, commonly indicated by abnormally elevated levels of cholesterol and triglyceride, is considered as a modifiable risk factor for cardiovascular disease due to its influence on atherosclerosis. Controlling the serum cholesterol and triglyceride within the normal range is a conventional strategy for the treatment and prevention of cardiovascular and cerebrovascular diseases. In a clinical study, breviscapine given to patients with hyperlipidemia at a dose of 25 mg per day for 2 weeks decreased the levels of total serum cholesterol, low density lipoprotein (LDL) cholesterol, and triglyceride, but increased the level of high density lipoprotein (HDL) cholesterol (Yu, 2011). Others have also observed lipid-lowering effects of breviscapine in elderly patients with hyperlipidemia (Wen and Ruan, 2004). The lipid-lowering effects of breviscapine were evaluated in patients with unstable angina pectoris and hyperlipidemia (Peng and Ye, 2011). No statins were used in the test or the control group. Breviscapine treatment produced statistically significant changes between the test and the control groups consistent with its lipid-lowering benefits. In addition, angina pain duration was reduced in the test group.

*2.3. Diabetic complications*

Diabetes mellitus results from the body's inability to produce or respond to the hormone insulin to meet the body's need, giving rise to abnormal metabolism of carbohydrates and elevated levels of glucose in the blood and urine. Persistent and uncontrolled high levels of blood glucose damage blood vessels and organ systems leading to diabetic complications, including cardiovascular disease, stroke, chronic kidney disease, peripheral neuropathy, foot ulcer, and retinopathy. In 2015, an estimated 425 million people had diabetes worldwide (International Diabetes Federation, 2018), representing 8.3% of the adult population (Shi and Hu, 2014). Diabetes at least doubles a person's risk of early death and about 1.5 to 5.0 million deaths each year result from diabetes (International Diabetes Federation, 2018; Roglic et al., 2005). The economic cost of diabetes in the U.S. in 2012 was estimated to be $245 billion, including $176 billion in direct medical costs and $69 billion in reduced productivity (Association, 2013). Diabetic complications, which are generally progressive and resistant to current drug therapy, are the major cause of the poor life quality, disability, mortality, and economic burden from diabetes (Papatheodorous, Papanas, Banach, Papazoglou, and Edmonds, 2016). Treating diabetic complications continues to represent a major challenge in drug therapy against diabetes. Breviscapine has received increasing attention as an addition to conventional medicines to treat diabetic complications, such as diabetic nephropathy and peripheral neuropathy, due to its broad pharmacological effects on the blood

circulation and blood vessels, as well as its anti-coagulation, anti-platelet, anti-inflammation, and anti-oxidant activities.

*2.3.1. Diabetic nephropathy*

Diabetic nephropathy is considered to be the most devastating complication from diabetes with regard to patients' quality of life and chance of survival, affecting 4.4% of the world's population by 2030 (Higgins and Coughlan, 2014; Lai et al., 2014). Current treatments for diabetic kidney disease provide only partial therapeutic effects and lesions in kidneys often progress to renal failure, requiring dialysis or transplant for survival. A number of clinical trials investigated the renal protective effect of breviscapine in diabetic nephrotic patients. A meta-analysis of these studies revealed the effectiveness of breviscapine in treating diabetic nephropathy (Liu et al., 2016). A total of 2260 patients in stages III-IV of diabetic nephropathy from 34 randomized and controlled studies were included, with 1158 patients receiving treatment with breviscapine plus conventional medicine (the treatment group) and 1102 receiving conventional medicine only (the control group). The dose of breviscapine injection ranged from 20 to 100 mg per day for a treatment duration of between 2 weeks and 1 month. The findings revealed that breviscapine significantly reduced a number of clinical parameters of kidney lesions compared with the control. These parameters include the 24-hour urine protein (standardized mean difference or SMD, −1.42; 95% CI, −1.83 to −1.02; $p < 0.001$); the urinary albumin excretion rate (weighted mean difference or WMD, −23.16; 95% CI, −37.20 to −9.12; $p < 0.001$); the serum creatinine level (WMD, −12.50; 95% CI, −18.16 to −6.84; $p < 0.001$); and the blood urea nitrogen or BUN level (WMD, −1.52; 95% CI, −2.25 to −0.78; $p < 0.001$). Breviscapine treatment also improved dyslipidemia that contributes to the progression of diabetic kidney lesions in the patients. Breviscapine reduced the levels of cholesterol and triglyceride, but increased the level of HDL cholesterol in these patients. Additionally, breviscapine reduced blood fibrinogen levels in patients with diabetic nephropathy, which is in accordance with breviscapine's effect on promoting fibrinolytic activity. Therefore, breviscapine protects against diabetic kidney complications by reducing urine proteins, improving renal functions, and adjusting dyslipidemia associated with diabetes.

*2.3.2. Diabetic peripheral neuropathy*

Diabetic neuropathy is the nerve-damaging disorder associated with diabetes, resulting from diabetic microvascular injury and, in some cases, macrovascular lesions as well. It affected about 132 million people in the world in 2010 and is considered to be one of the most common complications and the greatest source of morbidity and mortality in diabetes (Vos et al., 2012). Diabetic neuropathy affects 25% of patients with diabetes and is implicated in 50–75% of nontraumatic amputations (Snyder, Gibbs, and Lindsay, 2016). Drug therapy of diabetic neuropathy is limited to relieving pain with tricyclic antidepressants, anticonvulsants, focal analgesics, and non-steroidal anti-inflammatory drugs. Vitamin B12 (methylcobalamin) is beneficial to improving the symptoms of diabetic neuropathy. Breviscapine in conjunction with vitamin B12 has been shown to be effective in relieving the symptoms and signs of diabetic peripheral neuropathy in a number of studies and a meta-analysis of these studies confirmed the claims (Zheng et al., 2015). A total of 17 clinical randomized and controlled studies were included in the meta-analysis with 1398 patients suffering from diabetic peripheral neuropathy. Among the patients, 718 were treated with breviscapine and vitamin B12 (the intervention group) and 680 with vitamin B12 alone (the control group). The patients received a controlled diet, exercise, and glucose-lowering therapy before the interventions. In most cases, the patients were treated continuously for 2–6 weeks. As there is no unified curative effect evaluation standard available for diabetic neuropathy, the evaluation index was chosen based on the selected trials in order to evaluate curative effects. There was no apparent heterogeneity among the groups ($P = 0.74$, $I2 = 0\%$). A fixed effects model was used for pooled analysis to obtain an OR of 5.01, 95% CI of 3.70–6.78, and a Z value of 10.44 ($p < 0.0001$). Thus, the overall effectiveness of breviscapine and vitamin B12 was significantly superior to that of vitamin B12 alone. Significant improvement in the nerve conduction velocity was observed in the intervention group, including: (a) the median motor nerve conduction velocity or MNCV, with WMD of 7.53, 95% CI of 4.65–10.42, and Z value of 5.11 ($p < 0.0001$); (b) the median sensory nerve conduction velocity or SNCV, with WMD of 4.98, 95% CI of 1.75–8.21, and Z value of 3.02 ($p < 0.003$); (c) the peroneal MNCV, with pooled OR of 6.20, 95% CI of 4.69–7.72, and Z value of 8.02 ($p < 0.0001$); and (d) the peroneal SNCV, with pooled OR of 4.06, 95% CI of 2.80–5.32, and Z value of 6.33 ($p < 0.0001$). During the 2 to 6 weeks of therapy, only mild side effects were reported, including mild headache, nausea, itching, and palpitation at injection, which resolved after discontinuation of therapy. Therefore, the combined therapy with breviscapine and vitamin B12 is safe and effective in treating diabetic peripheral neuropathy.

*2.4. Other therapeutic effects*

*2.4.1. Glaucoma*

Glaucoma is a progressive optic neuropathy with characteristic pathologic alterations in the optic nerve head, resulting in loss of the visual field. The major detrimental effect of glaucoma is the destruction of retinal ganglion cells. Therefore, in addition to managing the intraocular pressure (IOP), neuroprotective therapies are needed. It has been shown that breviscapine could improve the activity of cytochrome oxidase in retinal ganglion cells and the optic nerve axoplasmic transport in rat models of acute IOP elevation (Zhu, Jiang, and Liu, 2000). A randomized and double-blind clinical trial was conducted to evaluate the visual field protective effect of breviscapine on 40 patients with a primary open-angle glaucoma, visual field defects, and postsurgical IOP of <18 mm Hg (Zhong, Xiang, Ye, Cheng, and Jiang, 2010). Two tablets of either breviscapine or placebo were given to the patients three times a day for 6 months. Breviscapine was found to significantly decrease the mean defect and the mean sensitivity between the values at pre-treatment and after 2, 4, and 6 months of treatment, compared with pre-treatment ($p < 0.05$), demonstrating that breviscapine exhibits a partial protective effect on the visual field of glaucoma patients with controlled IOP.

## 3. Pharmacokinetics

Scutellarin exhibits unusual pharmacokinetic behaviors in humans and animals. Like many plant-derived flavonoid glucuronides, scutellarin has a low solubility in body fluids, an unfavorable bioavailability, and a short half-life in mammalian systems. The bioavailability of scutellarin is exceptionally low, as evidenced by the very low plasma concentration of scutellarin after an oral dose. In a study on 20 healthy volunteers receiving an oral dose of 60 mg scutellarin, the parent drug could hardly be detected in the plasma with a mean maximal concentration ($C_{max}$) of <5.0 ng/mL, and its plasma concentration–time curve was rather atypical (Chen et al., 2006). In animals, the oral bioavailability of scutellarin was found to be merely 0.40% and 10.67% in dogs and rats, respectively (Ge et al., 2003; Huang, Weng, Huang, Ji, and Chen, 2005). These findings indicate that scutellarin may have a low absorption rate from the intestine and/or it undergoes a substantial metabolic change before reaching the systemic circulation.

While the serum concentration of scutellarin in humans is very low, that of its isomeric metabolite isoscutellarin (scutellarein-6-*O*-glucuronide, S-6-G) (Fig. 1c) is significantly higher, with a mean plasma concentration exceeding that of scutellarin by approximately 30-fold (Chen et al., 2006). This finding suggests that a regioselective mechanism exists in the intestine and/or liver to result in an isomeric switch from scutellarin to isoscutellarin. Similar to humans, rats have a low bioavailability for scutellarin and a high plasma exposure of isoscutellarin. The rat plasma scutellarin to isoscutellarin ratio is about 1.5 to 1 in the

systemic circulation, even though the mesenteric blood scutellarin to isoscutellarin ratio is as high as 15:1 after an oral dose of scutellarin (Gao et al., 2011). For these reasons, rats are considered as a preferred animal model for analyzing scutellarin pharmacokinetics, as the model better mimics the pharmacokinetic behaviors of scutellarin in humans. Besides isoscutellarin, the 6,7-diglucuronide of scutellarein (S-6,7-diG) (Fig. 1d) is another major metabolite excreted in the bile and the urine in both humans and rats (Gao et al., 2011, 2012).

The serum concentrations of scutellarin and its major metabolites also exhibit large variations among individuals in human volunteers and experimental animals. For instance, the $AUC_{0-\infty}$ (area under the curve from time 0 to the last measurable concentration) and $C_{max}$ values of the major metabolite isoscutellarin in the blood of 20 healthy Chinese subjects were 464.0 ± 154.0 and 87.01 ± 29.14, respectively. Some individuals exhibited double peaks for serum scutellarin and isoscutellarin (Chen et al., 2006; Ju et al., 2005). There were also considerable gender differences in the pharmacokinetic parameters of scutellarin in rats in which male rats appeared to have a lower absorption but higher clearance rate than female rats (Xing et al., 2011).

Considerable progress has been made in understanding these unique pharmacokinetic properties of scutellarin, especially the absorption and first-pass effect in the intestine, first-pass effect in the liver, regioselectivity, urinary excretion, enterohepatic circulation, and distribution of scutellarin in the brain. These aspects of scutellarin pharmacokinetics are discussed in more detail below.

### 3.1. Absorption, metabolism, and transport in the intestine

Scutellarin is poorly absorbed in the intestine, which is believed to be a major contributing factor to its low bioavailability from an oral exposure (Cao, Zhang, Guo, and Ping, 2008; Gao et al., 2011; Hao et al., 2005; You et al., 2010). Scutellarin has a pKa value of 2.75 and is mostly ionized at pH 6 to 8 in the intestine, resulting in a low lipophilicity (log D $> -4$) and poor membrane permeability. This notion negates a direct, passive diffusion of scutellarin as a major mechanism of absorption in the intestine. Indeed, several lines of evidence support that scutellarin is mainly absorbed in the intestine in the form of its aglycone, i.e., scutellarein, which is formed in the intestinal lumen through the hydrolysis of scutellarin by the β-glucuronidases of the intestinal microflora (Gao et al., 2011; Wang, Ao, Qian, and Zheng, 2011). First, scutellarin can hardly pass through a Caco-2 cell monolayer, an in vitro model of the human small-intestinal mucosa used for predicting the absorption of orally administered drugs. On the other hand, scutellarin is readily hydrolyzed into its aglycone in rat intestine, which exhibits a 15-fold higher permeability across the Caco-2 monolayer than scutellarin and can diffuse through the intestinal epithelial membrane effectively. Second, infusion of either scutellarin or its aglycone into a ligated rat intestine results in similar metabolite profiles and similar scutellarin/scutellarein ratios in the mesenteric plasma, suggesting a common mechanism(s) of intestinal absorption for the two compounds. Third, infusion of the aglycone produces higher amounts of metabolites in the mesenteric plasma than infusion of scutellarin, even at a four-fold lower concentration than scutellarin, suggesting that scutellarein, not scutellarin, is directly involved in the absorption.

Although scutellarin is likely to be absorbed in the form of scutellarein, no aglycone is detected in the portal vein plasma after an oral dose of scutellarin. Therefore, the aglycone absorbed must have undergone extensive biotransformation upon entering the intestinal epithelia and before being transported into the mesenteric blood. After the infusion of scutellarin or scutellarein, the mesenteric plasma predominantly contains monoglucuronides scutellarin and isoscutellarin, with a trace amount of 6,7-diglucuronide. However, the 6,7-diglucuronide becomes the predominant component in the bile, urine, and systemic plasma. Therefore, it was concluded that, in the intestinal epithelia, the aglycone scutellarein is mostly glucuronidated into monoglucuronides scutellarin and isoscutellarin, which upon entering hepatocytes, are further glucuronidated into 6,7-diglucuronide that is released into the bile and the plasma.

Glucuronidation of scutellarin aglycone in the intestinal epithelium is catalyzed by the microsomal enzyme uridine 5′-diphospho-glucuronosyltransferase (UGT) in rats and humans with high efficiency. The $V_{max}/K_m$ values for the total glucuronidation by the rat and human intestinal microsomes are comparable to each other, i.e., 1924 and 1498 $\mu l \cdot min^{-1} \cdot mg\ protein^{-1}$, respectively. As such, the intestine is believed to be the primary site for the formation of scutellarin and isoscutellarin after the absorption of aglycone into intestinal epithelia in both rats and humans (Gao et al., 2012). Most UGT enzymes (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B15, and UGT2B17), except for UGT2B7 that catalyzes the formation of isoscutellarin, exhibit a higher catalytic efficiency for the formation of scutellarin than isoscutellarin, though the formation of scutellarin in human intestinal microsomes is lower than that in rat intestinal microsomes. Both scutellarin and isoscutellarin can be further metabolized into scutellarein-6,7-diglucuronide, with a higher efficiency for isoscutellarin than for scutellarin. For instance, UGT1A1 and UGT1A8 exhibit a higher degree of selectivity toward isoscutellarin, whereas UGT1A10 has a selectivity for scutellarin, but with lower catalytic efficiency. Even though UGT1A10 is highly expressed in the intestine, glucuronidation of isoscutellarin is still favored over scutellarin in human intestine, because of the low catalytic efficiency of UGT1A10. Therefore, scutellarin is formed from scutellarein more easily but is metabolized into 6,7-diglucuronide more slowly than isoscutellarin. Based on these findings, it was concluded that the metabolism of scutellarin in the intestinal epithelium would not account for the conversion of scutellarin to isoscutellarin reflected in the plasma.

Both scutellarin and isoscutellarin formed in the intestinal epithelium are transported to the mesenteric blood through transporters, such as the multidrug resistance-associated protein (MRP) 3 located on the basal lateral membrane of enterocytes; alternatively, the monoglucuronides are secreted into the intestinal lumen via MRP2 and the breast cancer resistance protein (BCRP) on the apical membrane of enterocytes. These efflux transporters do not show sufficient selectivity between scutellarin and isoscutellarin and thus, would not explain the extremely low scutellarin to isoscutellarin ratio in the human plasma.

The intestinal absorption, hydrolysis, glucuronidation, and transport into the mesenteric blood of scutellarin is analogous to the fate of baicalin, a structural analog of scutellarin without the 4′-hydroxyl group (Fig. 1e), which, along with scutellarin, is a major bioactive component of *Scutellaria baicalensis* Georgi, a widely used Chinese herbal medicine. Baicalin is absorbed in the intestine in the form of its aglycone baicalein. Baicalein is then conjugated with glucuronide in the intestinal epithelium and the glucuronide conjugates are secreted into the mesenteric blood and the intestinal lumen by transporters MRP3 or MRP2/BCRP, respectively (Akao et al., 2000; Xing, Chen, and Zhong, 2005; Zhang et al., 2007).

### 3.2. Hepatic uptake, metabolism, and excretion into the bile

The liver plays a critical role in the metabolism and disposition of most foreign, as well as many endogenous, chemicals (Lee, Aizawa, Gan, Prakash, and Zhong, 2014). The mesenteric blood scutellarin and isoscutellarin are taken into hepatocytes through uptake transporters, mostly the organic anion-transporting polypeptides (OATPs), when passing through the hepatic sinusoids. Among the OATPs, OATP2B1 was found to be the major transporter for hepatic uptake of scutellarin and isoscutellarin. Notably, OATP2B1 exhibits a much higher substrate affinity and capacity for scutellarin than for isoscutellarin (Gao et al., 2012). The $K_m$ value for the transport of scutellarin by OATP2B1 was very low, approximately 1/24 of that for isoscutellarin, and the $V_{max}/K_m$ ratio of scutellarin is 4.3 times higher than that of isoscutellarin. Therefore, OATP2B1 is a high affinity and high-capacity uptake

transporter of scutellarin. Based on this notion, it was concluded that OATP2B1 plays a key role in the hepatic elimination of scutellarin and isoscutellarin from the blood; moreover, as it takes up scutellarin from the blood much more efficiently than isoscutellarin, it is likely to be a key determinant for the much lower systemic concentration of scutellarin than isoscutellarin in humans (Gao et al., 2012).

Upon entering hepatocytes, scutellarin and isoscutellarin are further metabolized into scutellarein-6,7-diglucuronide by UGT1A1 and UGT1A8. The diglucuronide formed in the hepatocytes is secreted into the bile via transporters MRP2 and BCRP. This hepatic glucuronidation of scutellarin and isoscutellarin is responsible for the high levels of the diglucuronide metabolite in both the bile and the urine. In rats, bile excretion of scutellarin and its metabolites can reach to a level equivalent to 44.2% of the administered dose, and include the following metabolites in a descending order of concentrations: 6,7-diglucuronide, scutellarin, methylated scutellarin, isoscutellarin, glucose conjugates (glucosidation products), the aglycone, and methylated aglycone (Gao et al., 2011).

The mono and di-glucuronides of scutellarein secreted from hepatocytes into the bile are largely excreted through feces; but some of them are converted back into scutellarein by the intestinal microflora and is re-absorbed in the lower intestine to serve as a new source of systemic scutellarin and isoscutellarin. This enterohepatic circulation of scutellarin and its metabolites may contribute to the development of the double peaks observed in human plasma after an oral exposure of scutellarin in certain individuals.

### 3.3. Renal excretion

In humans, isoscutellarin and the 6,7-diglucuronide of scutellarein are the major metabolites excreted in the urine. In rats, excretion of scutellarin is higher than that for isoscutellarin, which reflects the difference between the plasma concentrations of scutellarin and isoscutellarin in rats (i.e., a 1.5 to 1 ratio) (Gao et al., 2011; Gao et al., 2012). Other metabolites detected in the urine include scutellarein and the methylation and glucose conjugate (glucosidation) products. The total urinary recovery of a dose is approximately 5.42% in rats (Gao et al., 2011; Liu et al., 2009). Given the prominent roles of MRP2 and BCRP transporters in renal tubular secretion and in the intestinal and hepatobiliary excretion of scutellarin and isoscutellarin, it is expected that these transporters play important roles in the renal excretion of the glucuronide conjugates of scutellarein, though research data on their renal excretion through transporters is currently scarce in the literature.

### 3.4. Cerebral distribution under I/R

The low absorption and low plasma concentration of scutellarin and the frequent interconversions among scutellarin, isoscutellarin, aglycone, and aglycone diglucuronide in different organs raise a question of whether a pathophysiological condition, such as I/R, in cerebrovascular and coronary artery diseases would alter the pharmacokinetic properties of scutellarin.

In a rat model of I/R injury via the middle cerebral artery occlusion (MCAO), rats were administered with the Xin-Shao formula extract containing scutellarin and paeoniflorin, both of which are major constituents of the herbal medicine *Paeonia lactiflora*, by intravenous injection at 1.25 g/kg body weight (Li et al., 2016). The mean residence time ($MRT_{(0-t)}$) and the terminal elimination half-life ($t_{1/2}$) of scutellarin in the MCAO group were significantly prolonged compared to those of the sham-operated group (0.42 ± 0.15 vs. 0.26 ± 0.09 min, $p < 0.05$; and 1.66 ± 0.68 vs. 0.81 ± 0.48 min, $p < 0.05$). On the other hand, the disappearance of the plasma scutellarin was slower in the MCAO rats than that in the sham-operated rats (terminal clearance: 0.97 ± 0.26 vs. 1.57 ± 0.60 $L \cdot min \cdot kg^{-1}$, $p < 0.05$). The prolonged presence and the reduced rate of clearance of scutellarin in MCAO rats is believed to be due to reduced expression of UGT1A9 and UGT1A1 that catalyze the glucuronidation of scutellarin in the liver under a cerebral I/R injury. There was also reduced clearance of plasma paeoniflorin due to reduced metabolism of the drug by cytochrome P-450 (CYP) 3A2 in the liver.

In the case of stroke, a drug must cross the blood brain barrier (BBB) to affect the lesions in the brain. The BBB is formed by the brain capillary endothelium and prevents about 100% of large-molecule neurotherapeutics and >98% of all small-molecule drugs from reaching the central nervous system (CNS) tissues. Whether scutellarin or a metabolite of scutellarin crosses the BBB to cause the therapeutic effect in the brain is an intriguing question. By comparing the neuroprotective effects of scutellarin and scutellarein on repeated I/R in rats, it was shown that scutellarein attenuated neuronal lesions better than scutellarin and the metabolic changes after ischemic injury returned to near-normal levels after scutellarein intervention, which demonstrates a better CNS protective effect from scutellarein than from scutellarin (Tang et al., 2014; Tang et al., 2015). Because scutellarein crosses membranous barriers more readily than scutellarin, scutellarein is likely to pass the BBB and reach the brain at a higher concentration than scutellarin, which would result in a better therapeutic effect. Studies that would quantify the cross-BBB distributions of scutellarin and scutellarein are needed in order to validate this notion in future studies.

Fig. 2 summarizes the pharmacokinetic behaviors and the mechanistic basis underlying the unique features of scutellarin in its absorption, metabolism, and disposition in humans and rats. It is expected these pharmacokinetic insights would facilitate the understanding of the pharmacology and guide the future development of scutellarin-based drugs.

## 4. Pharmacological effect

A large effort was made to document and understand the pharmacological effects of scutellarin in experimental systems during the past three decades. In particular, a number of recent studies provided quantitative measurements of the therapeutic effects of scutellarin and related drugs on cerebral ischemic stroke, coronary heart disease, and diabetic complications in animal models. Experimental data also support the beneficial effects of the drugs in the treatment of several other disease conditions, such as cancer, neurodegeneration, and glaucoma. These studies validated the known clinical effects of the drugs, expanded their pharmacological profile, and provided mechanistic insights into the therapeutic effects of scutellarin.

### 4.1. Anti-ischemic stroke

Ischemic stroke constitutes >80% of all cerebral strokes and results in high incidences of mortality and paralysis (Donnan, Fisher, Macleod, and Davis, 2008). Ischemic strokes are caused by the occlusion of a cerebral blood vessel by a thrombus or embolus, leading to the rapid development of an ischemic infarct, accompanied by necrosis of neurons, glial reaction in the affected area, BBB disruption, and cerebral edema. Within the ischemic injury, the core of the infarct defines the area where the blood supply is nearly completely depleted, whereas the penumbra that surrounds the core retains a certain level of blood flow through collateral blood supply from surrounding arteries. A therapeutic goal is to rescue the affected neurons in the penumbra, which may be achieved through therapeutic interventions that reduce the infarct area and boost the recovery.

A number of studies demonstrated the efficacy of scutellarin in protection against cerebral ischemic injury in rats induced by MCAO, with or without reperfusion. In these MCAO models, a dye, such as triphenyl tetrazolium chloride (TTC), is used to stain the healthy brain tissue in red color, leaving the infarct area unstained with a white or pale appearance, which can be quantified as the infarct volume or percentage of infarction in the ipsilateral hemisphere in volume. In one study, brain I/R injury was created by MCAO for 2 h followed by reperfusion for 24 h

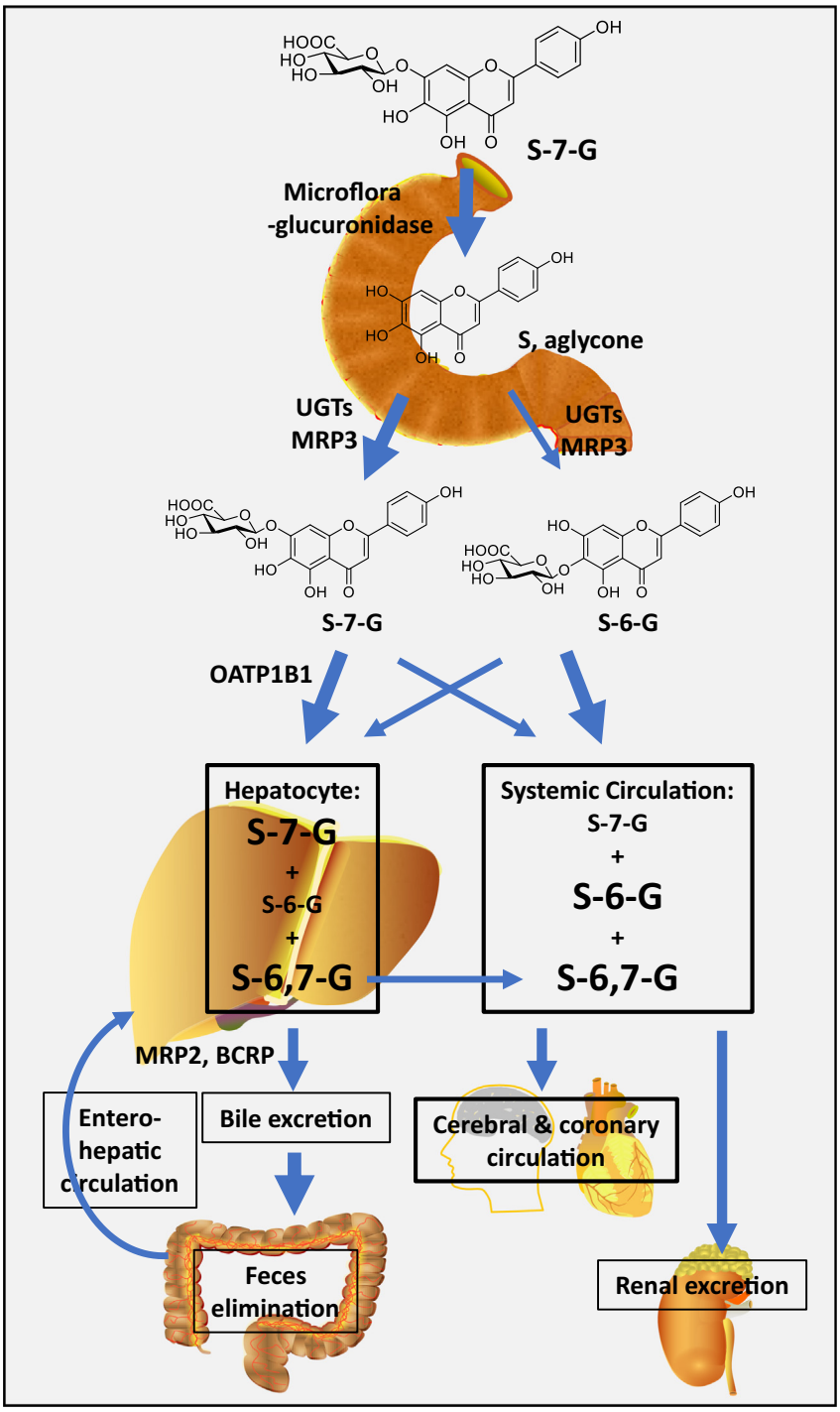


**Fig. 2.** Metabolism and disposition of scutellarin in humans. Orally administered scutellarin is rapidly hydrolyzed by intestinal microfloral β-glucuronidase into scutellarein, which is absorbed into enterocytes by passive diffusion and is re-conjugated with glucuronide to become scutellarin and isoscutellarin by UGTs at a ratio of ~2.7 to 1. Scutellarin and isoscutellarin are secreted into the intestinal lumen via MRP2 and BCRP or into the mesenteric blood via MRP3. The uptake of scutellarin and isoscutellarin into hepatocytes is mediated through OATP2B1 that has a 4.3-fold higher efficiency for scutellarin than for isoscutellarin, which may account for the much lower systemic blood concentration of scutellarin than that of isoscutellarin. Hepatic scutellarin and isoscutellarin can be secreted into the bile or converted to scutellarein-6,7-diglucuronide that is also secreted into the bile or released into the systemic circulation. Plasma isoscutellarin and the 6,7-diglucuronide are the major metabolites excreted through the urine. Intestinal scutellarin and its metabolites can be excreted through feces or are reabsorbed via the enterohepatic circulation. To reach the ischemic lesions in the brain, scutellarin or its metabolites must pass through the BBB, the mechanism of which is currently unclear, although scutellarein has been proposed to be a metabolite that passes through the BBB.

(Hu, Zhou, Hu, and Zeng, 2005). Rats receiving MACO and reperfusion had an infarct area of 31.26 ± 6.02%, a neurological score of 2.92 ± 1.27 (on a 5-point scale), and a significantly increased BBB permeability, compared with sham control. Pretreatment of the rats with scutellarin at 25, 50, and 75 mg/kg by intragastric administration (i.g.) for seven days reduced the infarction to 25.63 ± 5.12%, 18.23 ± 3.63%, and 9.24 ± 4.11%, respectively. Accordingly, I/R-induced neurologic deficits were reduced significantly. At the 50 and 75 mg/kg doses, scutellarin pretreatment reduced BBB permeability to near the sham control level. This neuroprotective effect of scutellarin against I/R injury was confirmed in a separate study at doses of 50 and 75 mg/kg in a similar I/R rat model (Zhang, Hu, Wang, Xu, and Zeng, 2009). Additionally,

scutellarin given by i.g. for 7 days decreased the infarct area, neurologic deficit, and cell apoptosis in a dose-dependent manner 24 h after a permanent MCAO injury without reperfusion (Wang et al., 2016).

The neuroprotective effects of scutellarin were compared with those of breviscapine, scutellarein, and edavarone—a free radical scavenger and anti-stroke agent. Scutellarin significantly reduced the infarct volume in a dose-dependent manner at 5, 15, or 50 mg/kg 24 h after intravenous administration (i.v.) following MCAO, with percentage of reduction reaching 25.4%, 32.3%, and 44.1%, respectively (Lin et al., 2007). At the same dose range, breviscapine reduced the infarct volume to a lesser extent than scutellarin. Others reported that breviscapine at 50 mg/kg by i.v. effectively reduced the infarct volume and improved the neurologic score after MCAO (2 h occlusion plus 24 h reperfusion) (Guo et al., 2014). By using a bilateral common carotid artery occlusion (BCCAO), a global ischemia was introduced in rat brain. Both scutellarin and scutellarein effectively attenuated the neural cell damage, reduced the cerebral water content, and improved a number of biochemical markers after an oral administration for 7 days in this BCCAO model; but scutellarein showed a better protective effect than scutellarin (Tang et al., 2014). In a separate study, scutellarin dose-dependently reduced the infarct area at 3 days after ipsilateral MCAO, which was more effective than edaravone; on the other hand, the combination of scutellarin and edaravone produced a synergistic anti-ischemic effect (Yuan et al., 2014). 6-*O*-Methyl-scutellarein (Fig. 1f) is a metabolite of scutellarin in vivo. In rats with repeated cerebral I/R injury, it was shown to significantly improve neuronal injury on behavioral, neurological, and histological examinations in a dose-dependent manner; moreover, it had a better protective effect than scutellarin in rat cerebral ischemia (Wu et al., 2017).

These studies consistently demonstrated the effectiveness of scutellarin, scutellarein, and breviscapine in treatment of ischemic brain injury, shown as reduction in the infarct volume and neurologic deficits, providing experimental data to support their therapeutic effects on patients with ischemic stroke. Scutellarin appears to be beneficial for the recovery of paralysis and other chronic neurologic deficits in patients during the chronic recovery phase of stroke. However, experimental evidence supporting such chronic effects of the scutellarin treatment remains to be obtained.

### 4.2. Cardiovascular protection

Scutellarin and breviscapine are widely used in the treatment of coronary artery disease or ischemic heart disease clinically, including angina pectoris and myocardial infarction. The drugs are also found to be beneficial in treating essential hypertension, cardiac arrhythmia, hyperlipidemia, and chronic heart failure. Increasing evidence indicates that some of the cardiovascular protective effects can be replicated in experimental systems, among which ischemia and I/R models for myocardial infarction are the best studied.

#### 4.2.1. Ventricular myocardial infarction

By ligature of the left anterior descending (LAD) branch of the coronary artery of rat heart, myocardial infarction is created in the left ventricle and the infarct is identified by staining the surrounding normal tissues with nitro blue tetrazolium in blue color or with TTC in red color. In one study, rats were given scutellarin at 5, 15, and 50 mg/kg or breviscapine at 50, 150, and 500 mg/kg by intraperitoneal (i.p.) injection, respectively, immediately after a LAD ligature (Lin et al., 2007). Four hours later, myocardial infarction was examined. Compared with the control (LAD ligature only), the scutellarin treatment reduced the average infarct size, expressed as the ratio between the infarct region weight and the left ventricle weight, significantly at 15 and 50 mg/kg doses from 24.9 ± 2.73 to 21.5 ± 3.74 and 18.9 ± 2.81, respectively. The percent reduction, calculated as (control infarct size − treatment infarct size) × 100 / control infarct size, was significantly increased accordingly. Under the same experimental condition, breviscapine at doses of 50 to 500 mg/kg did not produce significant effects compared with control. It is currently unclear why breviscapine failed to produce similar effects to those of scutellarin at doses 10 times higher than those of scutellarin in this model. The protective effect of scutellarin against myocardial I/R injury was demonstrated in a separate study (Li et al., 2015). Ischemia was introduced in the left ventricle by the closure of the LAD for 40 min, followed by reopening of the LAD to create reperfusion for 120 min, before the heart was taken for staining with TTC. No injury was found in the sham-operated heart, but myocardial I/R induced lesions in about 20.6% of the left ventricle. Treatment with scutellarin by i.v. at a dose of 45 mg/kg reduced the lesion area to about 15.4% and at 90 mg/kg, to about 13.2%.

In a separate study, the ventricular myocardial I/R injury was induced by a 30 min transient vessel occlusion of the LAD followed by 3 h reperfusion. The findings revealed that breviscapine significantly reduced the myocardium infarct size and the production of cardiac troponin or cTnI in the serum—a sensitive and specific marker of myocardial injury (Wang, Ji, Liu, Jing, and Lou, 2015). This result confirms that breviscapine can provide a significant protection against myocardial I/R injury in rats. Others have shown that breviscapine significantly reduced the levels of inflammatory cytokines and chemokines that were elevated due to left ventricle I/R injury in rats (Gong, Du, and Yuan, 2013; Wang, Ji, Chen, Wu, and Wang, 2013) and rabbits (Zhao, 2010), supporting a protective effect of breviscapine against myocardial infarction.

#### 4.2.2. Hypertension

The anti-hypertensive effect of scutellarin was investigated in a rat model of hypertension caused by renal artery constriction according to a 2-kidney, 2-clip, and 8-week procedure (Chen et al., 2013). Scutellarin was given to rats with the systolic blood pressure (SBP) above 140 mm Hg but without stroke symptoms at 5 mg/kg per day (low dose group) or 20 mg/kg per day (high dose group) by i.g., daily for 2 weeks. Sham-operation and saline were used as controls. The baseline SBPs were similar among the groups. No significant difference in SBPs was found before treatment among the groups. Compared with the saline group, scutellarin significantly reduced SBP in a dose-dependent manner, by 11.5 ± 6.5 mm Hg, i.e., from 180.9 ± 6.2 to 169.1 ± 7.1 mm Hg, for the low dose group, and by 17.2 ± 7.4 mm Hg, i.e., from 178.8 ± 6.7 to 161.2 ± 9.9 mm Hg, for the high dose group. This anti-hypertensive effect of scutellarin was associated with the attenuation of hypertension-induced expression of the Toll-like receptor 4 and the nuclear factor κ-light chain-enhancer of activated B cells (NF-κB) in the brain.

#### 4.2.3. Cardiac arrhythmia

The anti-arrhythmic effect of scutellarin was examined on ventricular arrhythmias in the hypertrophic myocardium of rabbit heart (Bo et al., 2011). It was shown that breviscapine diminished the transmural repolarization dispersion and reduced the incidence of early depolarization and torsades de pointes, leading to decreased incidence of ventricular arrhythmias in the hypertrophic heart.

#### 4.2.4. Hyperlipidemia

Breviscaine was shown to reduce blood lipid levels in diabetic rats. In one study, breviscapine inhibited the progression of intimal hyperplasia and atherosclerosis but did not decrease the level of serum cholesterol (Lou and Liu, 2009). A rat atherosclerotic model induced by high fatty diet combined with immunologic injury was used to evaluate the effect of scutellarin on blood lipid levels and aorta atherosclerotic lesions. Scutellarin at 10 and 20 mg/kg increased the serum superoxide dismutase (SOD), nitric oxide (NO), and HDL cholesterol, while decreasing the serum malondialdehyde (MDA), triglycerides (TG), and total cholesterol (TC). These findings indicate that scutellarin has antioxidant activities, improves dyslipidemia, and suppresses the aggravation of aorta

atherosclerosis in rats (Zhang et al., 2017). Overall, the lipid-lowering effect of scutellarin remains to be established experimentally, even though it has been observed in some clinical and to a much less extent, laboratory, studies.

### 4.3. Protection against diabetic complications

#### 4.3.1. Diabetic nephropathy

Diabetic nephropathy is a progressive renal disease, resulting from damage to the capillaries of the kidney glomeruli from high blood glucose and characterized by the nephrotic syndrome and diffuse scarring of the glomeruli, leading to renal failure. It is believed that hyperglycemia induces the development and progression of diabetic nephropathy through metabolic derangements, i.e., oxidative stress, polyol formation, accumulation of advanced glycation end products, and activation of mitogen activated protein kinase (MAPK) signaling, as well as hemodynamic alterations, such as hypertension (Brownlee, 2001). Breviscapine has been shown to be clinically beneficial in treating patients with diabetic nephropathy. Experimentally, it was shown that breviscapine given to rats at a dose of 20 mg/kg per day for 8 weeks can ameliorate the diabetic renal injury induced by streptozotocin (STZ), a diabetogenic agent, by inducing damage to pancreatic islet β cells (Qi et al., 2006). The breviscapine treatment markedly inhibited the increase of albuminuria, glomerular hypertrophy, and tubulointerstitial injury without modifying the mean arterial blood pressure and creatinine clearance. Breviscapine also provided superior renoprotective effects when combined with the ACE inhibitor enalapril in treating diabetic nephropathy in rats induced by STZ (Xu et al., 2013). The increased rate of urinary albumin excretion and kidney pathologic injury were attenuated by enalapril or breviscapine, but more effectively by the combined treatment with enalapril and breviscapine together. This improved therapeutic effect of the combined treatment with both drugs correlated well with a synergistic suppression effect between the two drugs on oxidative stress, the activity of protein kinase C (PKC), and the expression of transforming growth factor β1 (TGFβ1), providing a mechanistic basis for the combined clinical treatment over monotherapy on diabetic nephropathy.

#### 4.3.2. Diabetic retinopathy

Diabetic retinopathy results from damage to the microvasculature in the retina from high glucose. It affects up to 80% of the people who have had diabetes for over 20 years and is a leading cause of blindness worldwide (Kertes and Johnson, 2007). Scutellarin is believed to be beneficial for the treatment of diabetic retinopathy. However, its low bioavailability and low water solubility have limited its clinical use in treating retinopathy. Recently, a novel intestine-targeted nanoparticle carrier with amphiphilic chitosan derivatives (Chit-DC-VB12) loaded with scutellarin was used to enhance the bioavailability of scutellarin and to evaluate its therapeutic effect on diabetic retinopathy in rats (Wang et al., 2017). Diabetes induced a disordered arrangement of the ganglion cells and the inner nuclear layer cells in the retinal cell layers, which was eased by treatment with scutellarin and more effectively with vitamin B12-modified amphiphilic chitosan derivatives of scutellarin. This improved therapeutic effect correlated with an improved blood flow velocity in the retina and a better resistivity index of the central retinal artery, in addition to the down-regulation of the expression of angiogenesis proteins in the retina.

### 4.4. Protection against neurodegeneration

In addition to the protective effects on cerebrovascular ischemic injury, scutellarin exhibited other neuroprotective effects, among which the protection against neurodegeneration has received increasing attention. Neurodegeneration is the progressive loss of the structure and function of neurons in the affected brain, which provides the underlying basis for many neurodegenerative diseases, exemplified by Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). Such diseases are generally not curable at the present time.

Using a mouse model of ALS induced by cuprizone—a copper chelator and an inducer of demyelination—at 8 mg/day through the diet for 6 consecutive weeks, it was found that scutellarin at a dose of 50 mg/kg/day for 10 consecutive days can alleviate the behavioral deficits and other deteriorating parameters of ALS (Wang et al., 2016). The scutellarin-treated group showed a significant improvement of motor function (increased time to fall) by rotarod testing at the end of the treatment. The myelin basic protein staining of the corpus callosum revealed decreased demyelination. TUNEL staining followed by Nestin or Sox2 staining revealed an increased number and decreased apoptosis of neuro stem cells in the subventricular zone of the lateral ventricles. This protective effect against ALS may involve the activity of scutellarin to promote neural stem cells to differentiate into myelin-producing oligodendrocytes, which are injured during the development of ALS.

The β-amyloid peptide (Aβ) denotes the polypeptide of 36–43 amino acids from the amyloid precursor protein. Aβ can aggregate to form oligomers that are crucially involved in the pathogenesis of AD, as they are the major component of the amyloid plaques found in the brains of AD patients. In an in vivo rat model, Aβ was injected into the lateral ventricle (10 μg each side) (Guo, Guan, Huang, Wang, and Shi, 2013; Guo, Guan, and Wang, 2011). Scutellarin (10 mg/2 mL) or piracetam (10 mg/2 mL)—a medication for dementia—was administered by i.g., each day for 20 consecutive days following the Aβ treatment. Exposure to Aβ significantly increased the escape latency period and the time for first platform crossing, but decreased the total number of platform crossings, as compared with the control and the sham-operated groups. Both the scutellarin and the piracetam treatments significantly reduced the escape latency period and the time to cross platform, but increased the number of platform crossings. These results suggest that scutellarin protects against the development of AD.

A rat model of ischemic vascular dementia induced by a permanent occlusion of the bilateral common carotid arteries was used to evaluate the protective effect of breviscapine against dementia (Xiong et al., 2006). Breviscapine at a dose of 2 mg/kg daily for 14 days improved the performance of the learning and memory functions of the rats in the Morris water maze test. Additionally, it decreased lipid peroxidation and the levels of free radicals, as well as pathological alterations, such as nuclear shrinking, cellular edema, and the irregular arrangement of the pyramidal layer in the hippocampal CA(1) region.

### 4.5. Anticancer effect

The anticancer effect of scutellarin was suggested by the observation that scutellarin at a concentration of 100 μM sensitized HCT116 human colon cancer cells to resveratrol and 5-fluorouracil-evoked apoptosis in a p53-dependent manner (Chan, Tan, and Lee, 2009). It was subsequently found that scutellarin induced the apoptosis of HepG2 cells, a human hepatocellular carcinoma (HCC) cell line, promising to be a new anticancer drug candidate against liver cancer (Wu et al., 2010). Scutellarin elicited the apoptosis of HepG2 tumor cells by inhibiting the STAT3 (signal transducer and activator of transcription protein 3) pathway (Xu and Zhang, 2013). A mouse orthotopic liver xenograft model was employed to follow-up on these anticancer effects of scutellarin on liver cancer in vivo by assessing its effect on the migration and invasion of human HCC cells (SK-Hep1) (Ke et al., 2017). The mice were injected with $2 \times 10^6$ SK-Hep1 cancer cells in Matrigel into the left lobe of the liver. The mice were randomized and injected with scutellarin (50 mg/kg/day) or vehicle (normal saline) by i.p. daily for 35 days. The metastasis of the cancer cells into the lungs and within the liver was determined histologically. While clearly observable metastatic nodules were present in the lungs of control mice, there was no observable cancer nodules in the lungs of mice treated with scutellarin. In the liver, fewer numbers of intrahepatic nodules were found in the

scutellarin-treated mice than in the control mice. These differences in the formation of tumor nodules between the two groups were confirmed microscopically and were statistically significant. Moreover, the SK-Hep1 cells appeared to be invasive with the formation of microsatellites in the control liver; but the tumor cells in the liver of the scutellarin group had complete boundaries. Additionally, the numbers of metastatic tumors with a dimension of >0.5 mm in the lungs and the liver of scutellarin-treated mice were significantly less than those in the control mice. Therefore, scutellarin inhibited the lung and the intrahepatic metastasis and the growth of implanted HCC in mice.

The development of oral cancer involves multi-step carcinogenesis, including transformation from hyperplasia to dysplasia and ultimately to carcinoma. A significant metabolic transformation was found in hamsters treated with 7,12-dimethylbenz(a)anthracene, a known carcinogen, prior to the formation of squamous cell carcinoma, including elevated glutaminolysis and glycolysis, and decreased cholesterol and myo-inositol metabolism (Wei et al., 2012). These metabolic transformations can be normalized or significantly attenuated by treatment with breviscapine or salvianolic acid B—a major antioxidant and free radical scavenging compound from the plant *Salvia miltiorrhiza* or Danshen in traditional Chinese medicine. In a xenograft model of tongue squamous carcinoma, the growth of xenograft tumors in nude mice was significantly inhibited by the administration of scutellarin (Li et al., 2013). Moreover, scutellarin inhibited the proliferation and induced the apoptosis of the tumor cells and modulated the expression of matrix metalloproteinase (MMP)-2 and -9, as well as integrin $\alpha_v\beta_6$, genes implicated in tumor growth and metastasis, at the mRNA and protein levels in vivo.

Lung cancer is the second most common cancer in the world and is rising rapidly in developing countries such as China. In an in vitro study, breviscapine significantly reduced the growth of the lung cancer cells A549 and NCL-H460 (Zeng and Cai, 2017). This anti-proliferation effect involved the up-regulation of microRNA-7 (miR-7), which suppresses the growth and induces the apoptosis of these cancer cells.

Lymphoma are the neoplasms of the lymphatic tissues and the main classes of lymphoma are the malignant neoplasms of the lymphocytes. Anti-lymphoma drug therapy continues to present a major challenge, even though cytotoxic therapy, immunotherapy, and molecularly targeted therapy have been used in the clinic to treat the disease. Thus, new anti-lymphoma drugs are much needed. Scutellarin was found to have an anti-lymphoma effect. Scutellarin diminished the proliferation of B-lymphoma Namalwa cells in vitro and inhibited the growth of lymphoma in Namalwa cell-xenotransplanted mice without causing apparent toxicity (Feng et al., 2012).

### 4.6. Anti-glaucoma effect

A rat model with experimentally elevated IOP was used to mimic glaucoma. In this model, a high level of IOP blocks or inhibits the optic nerve axoplasmic transport, which can be measured by labeling the retinal ganglion cells with horseradish peroxidase that can be quantified. By using this model, the protective effect of breviscapine on glaucoma was evaluated at 15 mg/kg per day by i.p. for 20 or 40 days (Zhu et al., 2000). It was found that, at Day 0 of acute IOP elevation, no labeled retinal ganglion cells were observed. After 20 days of acute IOP elevation, the labeled retinal ganglion cell density was significantly increased by breviscapine compared with the control ($749 \pm 294/mm^2$ versus $423 \pm 220/mm^2$). After 40 days of acute IOP elevation, the density was further increased by breviscapine compared with control ($1048 \pm 393/mm^2$ versus $610 \pm 315/mm^2$). These findings indicate that breviscapine can improve the optic nerve axoplasmic transport that is blocked by an acute elevation of IOP in rats. This study confirms the protective effect of breviscapine against glaucoma observed clinically and provides an experimental approach to studying the therapeutic effect of scutellarin against glaucoma.

## 5. Mechanism, signaling pathways, and molecular targets

Elucidating the mechanism of action and identifying the targeting pathways and molecules of herbal drugs are necessary and critical for improvement of their therapeutic use. However, these wishful expectations remain a major challenge for most herbal medicine-derived therapeutics, despite enormous efforts made. In part, this is due to the fact that most herbal medicines evolve through experience rather than rational drug design and were sometimes based on abstract concepts, such as the "tonifying chi" theory used in traditional Chinese medicine. Moreover, clinically effective herbal preparations generally contain a mixture of known and unknown components that may all contribute to the therapeutic effect of the herbal medicine by affecting multiple drug targets. In this section, we discuss major mechanisms and pathways that have been implicated in the pharmacologic effects of scutellarin.

### 5.1. Scutellarin and I/R injury

An I/R injury is the tissue damage caused when blood supply returns to the tissue after a period of ischemia. Medical conditions characterized by I/R injury are among the most frequent causes of debilitating disease and death, exemplified by ischemic stroke and myocardial infarction. In these scenarios, the treatment of choice to reduce acute ischemic injury and infarct size is a timely and effective redelivery of the blood to lesioned area by way of thrombolytic therapy or surgical intervention, such as the primary percutaneous coronary intervention in the case of cardiac ischemic injury. However, this process of reperfusion can itself induce cell and tissue injury, known as reperfusion injury, for which there is no effective therapy (Hausenloy and Yellon, 2013).

The pathophysiology of I/R injury is complex (Kalogeris, Baines, Krenz, and Korthuis, 2012), as summarized in Fig. 3. During ischemia, the intracellular ATP level and pH value decrease as a result of anaerobic metabolism and lactate accumulation from hypoxia. As a result, the ATPase-dependent ion transport mechanism becomes dysfunctional, causing intracellular calcium overload, cell swelling, and cell death. Upon reperfusion, the oxygen level is restored, but a surge in the generation of reactive oxygen species (ROS) would occur and inflammatory cells, such as neutrophils, would infiltrate the ischemic tissue, all of which would exacerbate the ischemic injury. In this manner, the pathologic events induced by I/R orchestrate a cascade of pathogenic processes that include calcium overload, oxidative stress, inflammation, endothelial dysfunction, vascular dysfunction, and activation of apoptotic, autophagic, and necroptotic programs to result in cell death.

Scutellarin has been shown to be effective or beneficial for the treatment of ischemic stroke and myocardial injury, which can be attributed in part to its ability to reduce I/R injury in many cases. Mechanistically, the protective effect of scutellarin against I/R injury is associated with its activities to suppress inflammation, oxidative stress, and cell death, and to improve the hemodynamic balance—such as blood viscosity and platelet aggregation—and the vascular endothelial and smooth muscle cell functions, as discussed below.

### 5.2. Anti-inflammation

Inflammation is an adaptive tissue response triggered by noxious stimuli and conditions, such as tissue injury and microbial infection. Inflammation involves the reactions of innate immune cells, blood vessels, and molecular mediators. At a basic level, the acute inflammatory response to tissue injury encompasses a coordinated delivery of blood plasma and leukocytes to the site of injury. The injured tissues release damage-associated molecular patterns or DAMPs that are recognized by macrophages and dendritic cells through their cell surface receptors, i.e., pattern recognition receptors or PRRs. This leads to a cascade of events including the secretion of a plethora of pro-inflammatory soluble mediators, the recruitment of inflammatory cells, endothelial responses,

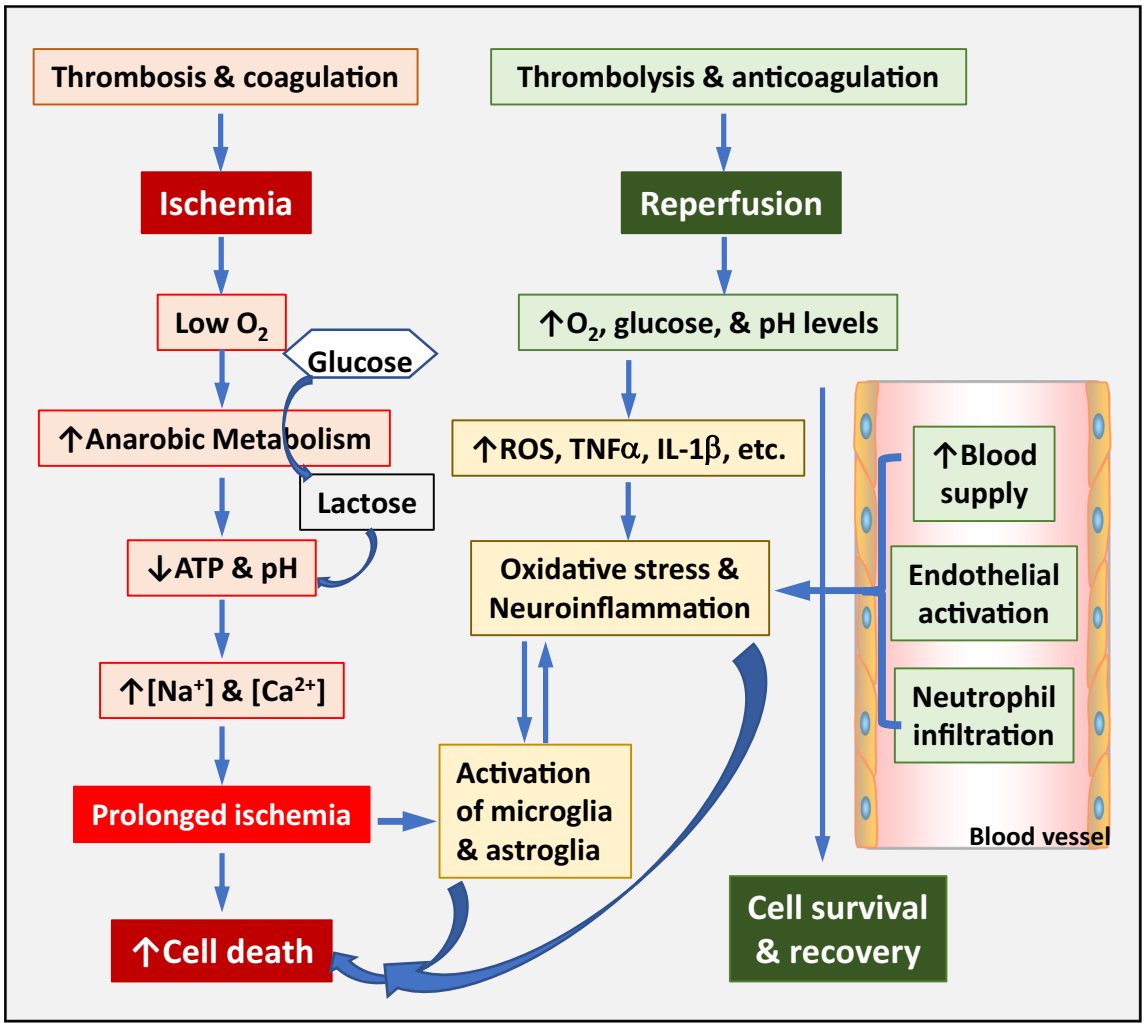


**Fig. 3.** Mechanisms of cerebral ischemic and I/R injury. During acute cerebral ischemia, the absence of oxygen switches the cell metabolism to anaerobic respiration, producing lactate and decreasing the ATP and pH levels. This leads to increased intracellular $Na^+$ and $Ca^{2+}$ overload, followed by mitochondrial lesions and eventually cell death if ischemia persists. During this process, a robust microgliosis occurs wherein reactive microglial cells remove damaged tissues by scavenging cell debris, but an exacerbated or prolonged microglial activation results in elevated production of ROS, RNS, and inflammatory mediators that are major mediators of the ischemic injury. The extent of an ischemic injury depends on the duration of ischemia and whether a collateral blood supply or reperfusion is provided. During reperfusion, the restored aerobic metabolism generates ROS in the neurons and glial cells. The vascular endothelial cells are activated and a large number of neutrophils are recruited from the circulation, both of which generate large amounts of ROS, RNS, and inflammatory cytokines and other soluble mediators. Oxidative stress and inflammation ensue to further exacerbate the tissue damage, which is known as I/R injury.

phagocytosis, and last but not least, tissue repair. The overall function of inflammation is to eliminate the insult, clear out damaged tissues, and initiate tissue repair. But an exacerbated or prolonged inflammatory response would worsen tissue damage and/or cause a chronic progression, leading to acute or chronic inflammatory diseases (Medzhitov, 2008). Inflammation contributes to the pathogenesis of ischemic lesions in both the CNS and peripheral organs. The mechanisms underlying the inflammatory development in these anatomically distinct systems appear to differ from each other.

*5.2.1. Neuroinflammation*

The CNS has been considered as an immunologically shielded site because peripheral immune cells are generally prevented from entering the CNS parenchyma by the BBB, a specialized barrier structure composed of astrocytes and endothelial cells that separates blood-derived immune cells from neural tissues in the CNS. Nonetheless, circulating immune and inflammatory cells may penetrate a compromised BBB and encounter neurons and glial cells to launch and propagate immune and inflammatory responses upon injury. Although neuroinflammation is generally initiated to protect the CNS from infectious and noxious agents, its effect may be toxic and widespread inflammation may cause extensive damage to CNS tissues (Gendelman, 2002). Acute inflammation usually immediately follows an injury to the CNS, and is characterized by inflammatory secretion, endothelial activation, platelet deposition, and tissue edema, whereas chronic inflammation typically reflects a sustained activation of glial cells and recruitment of other chronic immune/inflammatory cells into the brain.

Microglial cells are the resident innate immune cells responsible for the immune surveillance in the CNS (Aguzzi, Barres, and Bennett, 2013). Upon exposure to a stimulus, microglial cells transform to become "activated" or "reactive" microglia that defend against the noxious insult by scavenging microbes and cellular debris, releasing neurotrophic factors, and promoting the resolution of inflammation. On the other hand, if persistently activated, reactive microglia release excessive amounts of proinflammatory cytokines and cytotoxic agents, such as NO, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and ROS, to cause neural damage. The cells also release glutamate—a neurotransmitter and an excitotoxic agent—to cause delayed neuron death. During stroke, a deficiency of blood supply to the brain rapidly leads to the development of ischemic infarction, accompanied by neuron degeneration and death, glial activation, BBB disruption, and cerebral edema. During this process, a robust microglial reaction develops to mediate many of the beneficial,

as well as pathologic, functions of neuroinflammation (Yuan et al., 2016).

Scutellarin inhibits microglia-mediated inflammatory functions. In one study, scutellarin was shown to attenuate lipopolysaccharide (LPS)-induced production of proinflammatory mediators, including NO, TNFα, IL-1β, and ROS, in cultured primary rat microglia or the BV-2 mouse microglial cell line (Wang et al., 2011), as well as activated microglia of rats subjected to MCAO (Yuan et al., 2014). Additionally, scutellarin enhanced the expression of nerve growth factors, a brain-derived neurotrophic factor, and the glial cell-derived neurotrophic factor in astrocytes under hypoxia/reoxygenation (Chai et al., 2013). It also improved the neurologic deficit and the neuronal maturation in rats with cerebral ischemia (Li, Lu, and Zhang, 2013). Scutellarin inhibited microglial migration in vitro and in vivo and promoted the formation of flattened and microspike projections by promoting the reorganization and stabilization of cytoskeletal dynamics in activated microglial cells, which supports the inhibition of microglial migration by scutellarin (Yuan et al., 2015).

In MCAO rats, astrocytes are activated and reactive astrogliosis takes place following microglial activation. Scutellarin increased the expression of the glial fibrillary acidic protein (GFAP) and nestin as well as proinflammatory mediators in reactive astrocytes during ischemic injury. Scutellarin appears to boost astrogliosis by increasing the expression of GFAP, TNF-α, IL-1β, and the inducible nitric oxide synthase (iNOS) via activated microglia, which suggests a cross-talk between microglia and astrocytes during neuroinflammation (Fang et al., 2015).

The pathways through which scutellarin inhibits microglia-mediated neuroinflammation during I/R injury have been examined. Scutellarin decreases the expression of NF-κB p65, a key transcription factor for the expression of major proinflammatory cytokines, signaling molecules, and enzymes, including TNF-α, IL-1β, iNOS, and cyclooxygenase-2, both in vitro and in vivo, such as in the MCAO rat model (Chen et al., 2013; Wang, Wang, et al., 2011; Yuan et al., 2015). The Notch signaling pathway is involved in neuronal development, postnatal brain functions, and the pathogenesis of CNS diseases (Artavanis-Tsakonas, Rand, and Lake, 1999). Relevant to CNS ischemia, the Notch pathway regulates microglia-mediated innate immune response in cerebral ischemia. Notch mRNA and protein were upregulated in activated microglia and Notch-1 antisense mice exhibited reduced microglial activation and reduced expression of proinflammatory cytokines in the ipsilateral ischemic cortex (Yao et al., 2013). Notch signaling was upregulated in primary microglia and BV-2 cells under hypoxia in LPS-stimulated BV-2 cells, and in vivo in MCAO rats (Yao et al., 2013; Yuan et al., 2015). Inhibition of Notch signaling prevented hypoxia-induced upregulation of NF-κB target gene expression in microglial cells in rats, indicating that Notch activation is upstream of NF-κB activation and regulates the functions of activated microglia (Yao et al., 2013). This notion was supported by a separate study in which scutellarin markedly decreased the expression of Notch 1 and various downstream proteins of Notch signaling in MCAO rats and in cultured cells; inhibition of Notch by scutellarin was also associated with the inhibition of the NF-κB and the STAT1 singling pathways (Yuan et al., 2015). Additionally, scutellarin increased the expression of Notch signaling proteins in reactive astrocytes both in MCAO rats and in astrocytes in vitro in a microglia-dependent manner (Fang et al., 2015). This observation is consistent with the finding that scutellarin promoted astrogliosis through its effect on activated microglia to boost tissue repair in the brain. Therefore, scutellarin appears to attenuate microglia-mediated neuroinflammation by modulating the Notch and possibly several other pathways.

The renin-angiotensin system plays multiple roles in cerebral vascular functions, including neuroinflammation and brain ischemic injury. In a recent study, scutellarin was given to rats by i.g. for seven days before a permanent cerebral ischemic injury was introduced by MCAO for 24 h (Wang, Ma, et al., 2016). Treatment with scutellarin dose-dependently decreased the neurologic deficit score, the infarct area, cellular apoptosis, and the morphological alterations induced by MCAO. Moreover, these protective effects were closely associated with the reduction of ACE and angiotensin II (Ang II) type 1 receptor (AT1R) expression as well as the levels of Ang II, TNF-α, IL-6, and IL-1β in ischemic brains. These findings indicate that scutellarin protects the brain from acute ischemic injury, which involves the inhibition of the ACE/Ang II/AT1R axis, the cerebral blood flow preservation, and the inhibition of proinflammatory cytokine secretion.

#### 5.2.2. Inflammation in cardiovascular disease

Inflammation is central to the development of cardiovascular diseases and their associated complications. During an acute myocardial infarction, local processes are first activated with the release of ROS and cytokines and the infiltration of circulating neutrophils and monocytes, leading to acute myocardial injury. Several remote sites are also involved, including the spleen and the bone marrow, via various signaling pathways to further activate the immune system and worsen the injury. Thereafter, a reparative phase ensues, which is predominantly mediated by monocytes and T-lymphocytes, resulting in tissue repair and recovery, with the upregulation of processes involved in angiogenesis and extracellular matrix deposition (Ruparelia, Chai, Fisher, and Choudhury, 2017).

The anti-inflammatory effect of breviscapine on myocardial I/R injury was evaluated in a New Zealand rabbit model. Treatment with breviscapine resulted in an apparent myocardial protective effect by reducing inflammatory lesions in the heart, compared with control. In this model, the treatment decreased the expression of myocardial TNF-α and NF-κB, indicating an inhibition of acute inflammation (Zhao, 2010). Breviscapine also attenuated inflammation and I/R injury by decreasing the expression of TNF-α and IL-6 in a rat model of cardiac I/R injury (Gong et al., 2013). Scutellarin also reduced the fibrotic change during the reparative phase after myocardial injury from either chemical induced lesion by isoprenaline or coronary artery occlusion, indicating that scutellarin has a protective effect against the chronic inflammatory lesions from myocardial infarction (Pan et al., 2011; Zhou et al., 2014).

### 5.3. Anti-oxidative effect

Reactive oxidants (ROS and reactive nitrogen species or RNS) are constantly generated in mammalian systems from internal metabolism and external exposures (Ma, 2013). Under physiologic conditions, ROS and RNS production serves useful purposes, such as functioning as signaling molecules to regulate cell division, inflammation, and immune functions, and are counterbalanced by elaborate antioxidant systems in the body. Uncontrolled production of oxidants results in oxidative stress that damages cells and contributes to disease development (Ma, 2010; Ma and He, 2012).

Oxidative stress plays a major role in the initiation and propagation of I/R injury. Ischemia first activates endothelial cells and resident innate immune cells, such as microglia and macrophages; both types of cells produce large amounts of ROS and RNS that trigger tissue damage and inflammatory response. Restored blood flow upon reperfusion reintroduces oxygen within cells and brings in circulating inflammatory cells and inflammatory mediators, which further increase ROS and RNS production and induction of iNOS, resulting in significant oxidative stress and damage. Moreover, the antioxidant defense mechanisms within the ischemic area are generally depleted or considerably weakened, further exacerbating oxidative stress. Thus, suppression of oxidative stress is commonly considered as an integral part of the therapeutic approach to I/R injury.

As a plant-derived flavonoid, scutellarin is an effective radical scavenger in vitro against ROS and RNS, such as hydroxyl radical, superoxide anion radical, and hydrogen peroxide (Liu, Yang, Zhou, and Xu, 2002). Scutellarin protected rat cortical synaptosomes from oxidative damage induced by incubation with xanthine and xanthine oxidase that

produce superoxide in vitro, at a concentration range of 25 to 100 μmol/L (Liu et al., 2003). Scutellarin suppressed the induction of iNOS, the production and release of NO, and the lipid peroxidation and cell death of cultured rat primary neuronal cells exposed to hydrogen peroxide (Liu, Yang, Tang, Liu, and Xu, 2005). Scutellarin also protected cardiomyocytes from I/R injury through its anti-oxidant effect. For instance, rat cardiomyoblast H9C2 cells were used to create an in vitro I/R injury model by exposing the cells to hypoxia plus glucose and serum-deprivation for 2 h, followed by 6 h recovery. Scutellarin dose-dependently suppressed the I/R-induced oxidative stress, indicated by reduced ROS production and lipid peroxidation, but elevated expression of the antioxidant enzyme SOD (Wang et al., 2016).

In a rat model of cerebral I/R injury (2 h ischemia by MCAO, followed by 24 h reperfusion), scutellarin was given to rats at 50 and 75 mg/kg i.g. for 7 days before ischemia. The treatment reduced the infarct size and improved the neurologic score and BBB permeability. These protective effects of scutellarin correlated with the induction of the endothelial NOS (eNOS) and the suppression of expression of iNOS (Hu et al., 2005). Induction of eNOS is associated with vasodilation, inhibition of platelet aggregation, and neutrophil adhesion, and therefore, is generally considered as beneficial; in contrast, induction of iNOS during ischemia is often cytotoxic. In a separate study, scutellarin by i.p. injection at doses of 20 and 60 mg/kg improved the neurologic score and diminished the percentage of the brain infarct volume in rat brain with a cerebral I/R injury via MCAO (Guo et al., 2011). Scutellarin significantly increased the activities of SOD and catalase and the level of glutathione in ischemic brain tissues, which enhanced the endogenous antioxidant functions. Pretreatment with scutellarin at 25, 50 and 100 μM protected neurons against lethal exposures, decreased the percentage of apoptotic cells, and inhibited ROS generation in primary cortical neurons with oxygen and glucose deprivation in vitro. These results suggest that the preventive and therapeutic potential of scutellarin in patients with cerebral I/R injury is in part attributable to the augmentation of the cellular antioxidant defense capacity.

Cerebral ischemic stroke may cause injury at distant sites, such as the liver. In a model of rat cerebral I/R (2 h ischemia followed by 3 h reperfusion)-induced liver injury, pretreatment with scutellarin at 50 and 75 mg/kg by i.g. for 7 days before I/R was found to significantly reduce the liver injury, as indicated by reduced activities of liver enzymes ALT, AST, and XOD in the serum that were significantly elevated in the control groups by I/R. These protective effects of scutellarin were associated with reduced lipid peroxidation, reduced NO levels in both the serum and liver, and elevated levels of SOD and glutathione peroxidase in the liver, by the scutellarin pretreatment (Yang, He, Lu, and Zeng, 2003).

Scutellarin also inhibited oxidative stress and ROS-stimulated cellular damage induced by Aβ in rat brain and in cultured neuronal cells as discussed in more details in previous sections (Guo et al., 2013).

### 5.4. Anti-apoptosis

In ischemia, many cells undergo programmed cell death known as apoptosis, which constitutes a major mechanism by which ischemia induces cell death. Ischemia and I/R can lead to the production of a range of apoptosis-inducing factors, among which, NO, ROS, and a rise in the intracellular calcium concentration may trigger the intrinsic pathway of apoptosis. Extracellular factors, such as TNF-α, activate the extrinsic pathway of apoptosis. Both pathways involve the activation of caspases, which leads ultimately to cell death.

An I/R injury in the brain (2 h ischemia by MCAO, followed by 22 h reperfusion) was found to induce an increase in DNA fragmentation (TUNEL assay) and a significant decrease in the nicotinamide adenine dinucleotide (NAD) level in rats, which correlated with the infarction and neurologic deficit. Pretreatment of the rats with scutellarin at 50 and 75 mg/kg by i.g. for 7 days before ischemia significantly reduced the infarct volume and the neurologic deficit, as well as the DNA fragmentation and NAD depletion in the brain. Additionally, the pretreatment reduced the over-activation of the poly (ADP-ribose) polymerase (PARP) and the translocation of the apoptosis-inducing factor (AIF) from the mitochondria to the nucleus (Zhang, Hu, et al., 2009). These findings implicate PARP-dependent mitochondrial dysfunction and the translocation of AIF in the anti-apoptotic activity of scutellarin. In an in vitro cardiomyocyte I/R injury model (Wang, Yu, et al., 2016), scutellarin reduced I/R-induced apoptosis, accompanied by improved mitochondrial membrane potential, decreased expression of pro-apoptotic molecules, Bax and caspase-3, and increased expression of pro-survival proteins, Bcl2, VEGF, MMP2, and MMP9.

As discussed in other sections, scutellarin also induces apoptosis in several cancer cell lines, thus promising to be an effective anticancer drug. Therefore, scutellarin appears to exhibit both apoptosis-suppressing and apoptosis-inducing effects in a cell type and context-dependent manner. The molecular basis for these seemingly opposing effects of scutellarin on cancer versus non-cancer cells is not clear. The rapid growth and division, a largely increased demand for energy and nutrients, and a highly dysregulated microenvironment of cancer cells may have sensitized the cells to apoptosis-inducing effects of scutellarin, though further studies are needed to verify this notion.

### 5.5. Anti-thrombosis and anti-coagulation

#### 5.5.1. Anti-thrombosis

Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. Platelet activation and aggregation are critical steps in thrombosis. The formation of a platelet plug at a site of atherosclerotic lesion rupture is the most common promoting factor for acute myocardial infarction and cerebral ischemic stroke (Michelson, 2010). Platelets are activated by a set of specific signaling factors that are agonists of their surface receptors, including thromboxane A2, ADP, and thrombin. Antiplatelet agents targeting the thromboxane A2 (aspirin) and the ADP (p2Y13 inhibitors clopidogrel and ticlopidine) pathways have been beneficial in reducing mortality and morbidity in myocardial infarction and stroke. However, the use of existing antiplatelet agents is often associated with increased risks of side-effects. Thus, more effective and safer antiplatelet drugs are needed.

While scutellarin and breviscapine are often prescribed with antiplatelet drugs in the treatment against stroke, coronary heart disease, diabetes, and hypertension, pharmacological studies demonstrate that scutellarin and scutellarein themselves are effective antiplatelet agents. For instance, *Erigeron breviscapus* flavones were shown to significantly inhibit the formation of thrombi induced by ADP, arachidonic acid, and platelet activating factor (Shen, Lei, Li, and Chen, 2000). Scutellarein could inhibit platelet aggregation in rats by reducing the platelet cytosolic free calcium concentration (Li, Xu, Li, Chen, and Ding, 2004). In another study, scutellarein inhibited platelet aggregation, prevented thrombosis, and improved hemorheologic parameters by reducing the ADP-induced platelet aggregation rate in rats (Song, Zhang, Ma, and Li, 2011). A recent study demonstrated that scutellarein inhibited the platelet adhesion and aggregation induced by thrombin, $U_{46619}$, and ADP in a concentration-dependent manner. It was also an effective inhibitor of PKC and exhibited a large inhibitory effect on ADP-induced platelet aggregation (Tian et al., 2016).

#### 5.5.2. Anti-coagulation

Coagulation is the process by which the blood changes from a liquid state to a gel, forming a blood clot. The dynamic interactions between the blood coagulation system and the anticoagulation and fibrinolytic systems determine the development of blood coagulation or bleeding. Breviscapine significantly delayed the coagulation time and the prothrombin time, inhibited platelet factor III activity, and decreased the euglobulin lysis time, thereby enhancing anticoagulation (Wang, Yang, Liu, and Tang, 2003).

### 5.6. Vasodilation and vascular protection

#### 5.6.1. Vasodilation

Vasodilation results from the relaxation of the smooth muscle cells in the blood vessel wall, which is critical for providing increased blood flow to where it is most needed. Vasodilation can be triggered by the release of a number of endothelia-dependent and non-endothelia-dependent factors via α or β-adrenergic receptors, the $Ca^{2+}$ channel, and the $Ca^{2+}$-dependent $K^+$ channel on the cell membrane under physiologic and pathophysiologic conditions. Scutellarin has been shown to relax norepinephrine-induced vasoconstriction in vitro in a concentration-dependent manner without affecting endothelial functions; this effect was associated with an inhibition of the receptor-operated calcium channel (Zheng, Gond, and Liang, 1998). Scutellarin at concentrations of 3, 10, 30, and 100 μM caused a concentration-dependent relaxation in both endothelium-intact and endothelium-denuded rat aortic rings pre-contracted with noradrenaline bitartrate ($IC_{50} = 7.7 \pm 0.6$ μM). This vasodilation effect by scutellarin did not involve potassium channels, muscarinic receptors, the nitric oxide pathway, or prostaglandins. Pretreatment with scutellarin decreased the tonic phase, but not the phasic phase of the noradrenaline bitartrate-induced tension increment. Scutellarin also alleviated the $Ca^{2+}$-induced vasoconstriction in $Ca^{2+}$-depleted and noradrenaline bitartrate-pretreated rings in the presence of the voltage-dependent calcium channel blocker verapamil, and inhibited the noradrenaline bitartrate-evoked intracellular calcium increase. Therefore, scutellarin can relax thoracic artery rings in an endothelium-independent manner, which may involve the inhibition of the extracellular calcium influx independently of the voltage-dependent calcium channel (Pan et al., 2008).

Scutellarin also induced the endothelium-dependent vasodilation, which was partly affected by inhibitors of NOS and guanylate cyclase in isolated mouse aorta (Yang, Lust, Bofferding, and Wingard, 2009). In a recent study, an endovascular perforation was used to induce subarachnoid hemorrhage in rats. Scutellarin was given to rats via an intra-cerebroventricular injection (50 μM, 100 mg/kg) immediately after subarachnoid hemorrhage. Scutellarin alleviated the angiographic vasospasm, improved neurological functions, and increased the expression of eNOS 48 h after subarachnoid hemorrhage (Li et al., 2016). Moreover, scutellarin upregulated the expression of the phosphorylated extracellular signal-regulated kinase (Erk) 5 or p-Erk5 and Krupple-like factor 2 at 48 h after subarachnoid hemorrhage, which was reversed by the Erk5 inhibitor XMD8-92, indicating that scutellarin may inhibit the vasospasm and the neurological deficits by modulating the Erk5-KLF2-eNOS pathway after subarachnoid hemorrhage.

#### 5.6.2. Vascular protection

The vascular endothelium plays multiple roles under physiologic conditions and damage to the endothelium contributes to various vascular dysfunctions, such as thrombosis, vasoconstriction, ROS production, and release of inflammatory cytokines. Breviscapine reduced the damage of TNF-α to cardiac microvascular endothelial cells by inhibiting the inflammatory response (Zhang et al., 2009). Breviscapine also inhibited ox-LDL-induced endothelial cell damage, possibly through its antioxidant effects and the inhibition of NF-κB activation (Chen, Ren, Sun, and Guo, 2015). Scutellarin protected vascular endothelial cells from I/R injury by increasing the expression of the vascular endothelial growth factor (VEGF) measured in human umbilical vein endothelial cells (Lin, Hua, Cai, and Yang, 2011).

The protective effect of scutellarin on coronary vascular endothelial cells after myocardial I/R injury was investigated in vivo in a rat model where ischemia was induced by LAD ligature for 40 min followed by reperfusion for 120 min (Li, Li et al., 2015). Scutellarin was given to the rats intravenously via the tail vein at 15 min before an I/R surgery until the end of reperfusion. Scutellarin at 45 and 90 mg/kg significantly reduced the left ventricular ischemic size and restored the endothelium-dependent vasodilation of isolated coronary artery rings. Protein kinase G (PKG) inhibitor Rp-8-Br-cGMP could ameliorate the protective effects at 50 mg/kg; and an increase in the phosphorylation of the vasodilator-stimulated phosphoprotein, a main substrate of PKG, was observed. Cultured human cardiac microvascular endothelial cells were used to study the molecular mechanism by which scutellarin modulates PKG-1α signaling. The findings suggest that PKG-1α plays an important role in the protective effects of scutellarin on the endothelial dysfunction induced by myocardial I/R injury.

The protective effect of scutellarin on cerebral vascular endothelial cells was investigated both in vitro in human brain microvascular endothelial cells with a hypoxia reoxygenation treatment, and in vivo in rats with cerebral I/R injury in which the right external carotid artery was ligated to cause cerebral ischemia for 1 h followed by reperfusion for 24 h through the right middle cerebral artery (Du et al., 2015). In cultured human brain microvascular endothelial cells, scutellarin at 0.1, 1, and 10 μM increased cell viability and mRNA, protein, and activity levels of PKG and the vasodilator-stimulated phosphoprotein against the hypoxia/reoxygenation injury. In the rat cerebral I/R model, scutellarin at 45 and 90 mg/kg by i.v. significantly reduced the ischemic size by partially restoring the endothelium-dependent vasodilation of the basilar artery. The PKG inhibitor Rp-8-Br-cGMPS at 50 μg/kg by i.v. reversed this protection by scutellarin in the I/R rats. Therefore, scutellarin protects against cerebral vascular endothelial cells by activating the endothelial PKG pathway.

Vascular smooth muscle cells migrate and proliferate to contribute to the development of atherosclerosis and hypertensive vascular lesions. For this reason, vascular smooth muscle cells become therapeutic targets of vascular diseases. Breviscapine was shown to reduce the proliferation of rat aortic smooth muscle cells, which may involve blocking the thrombin receptor gene expression (Hou, Zhang, and Zhang, 2009). A separate study also demonstrated that breviscapine inhibited vascular smooth muscle cell proliferation and prevented atherosclerosis, which were, in part, mediated by modulating the NF-κB activity of the cells (Pang, Pan, Long, Qin, and Ya-Lin, 2004).

### 5.7. Myocardial protection

Myocardial injury from I/R, diabetic high glucose, and hypoxia may evolve into chronic cardiac hypertrophy and myocardial fibrosis. Breviscapine was shown to protect against myocardial injury and its chronic progression. Breviscapine significantly reduced LDH leakage, the intracellular free Ca2+ concentration, and apoptosis of cardiomyocytes exposed to hypoxia (Li et al., 2004). Breviscapine protected against cardiac hypertrophy by disrupting the PKC-α-dependent ERK1/2 and PI3K/AKT pathway in cardiac myocytes and in mouse heart (Yan et al., 2010). Scutellarin was also shown to protect against isoprenaline-induced myocardial fibrosis via its effect on the cardiac endothelial mesenchymal transition and the Notch pathway (Zhou et al., 2014).

### 5.8. Anticancer effect

Scutellarin exhibits anticancer effects on cancer cell lines derived from tumors of the liver, lung, colon, and lymph tissues (Chan et al., 2009; Feng et al., 2012; Wu, Fan, et al., 2010; Xu and Zhang, 2013; Zeng and Cai, 2017), as well as in xenograft tumors of the liver and tongue cancers (Ke et al., 2017; Li, Huang, et al., 2013). In these scenarios, scutellarin induced or promoted prominent apoptosis of the cancer cells, but the mechanism by which scutellarin induces apoptosis in these cells appears to vary considerably.

Scutellarin markedly inhibited the proliferation and induced significant apoptosis of HepG2 cells, which were attributed to the down-regulation of STAT3 transcriptional targets Bcl-XL and Mcl-1 (Xu and Zhang, 2013). In a study with lung cancer cells A549 and NSCLC (non-small cell lung cancer), breviscapine significantly reduced growth and increased apoptosis of A549 and NCLH460 cells in a concentration- and time-dependent manner (Zeng and Cai, 2017). Breviscapine-

treated A549 cells showed increased levels of Bax and microRNA-7 (miR-7) and a decreased level of Bcl-2. The up-regulation of miR-7 enhanced the sensitivity of NSCLC cells to breviscapine by suppressing cell proliferation and promoting cell apoptosis, whereas an inhibition of miR-7 reversed the anti-proliferative and pro-apoptotic effects of breviscapine. Pre-treatment with miR-7 mimics enhanced the breviscapine-mediated down-regulation of Bax/Bcl-2 in NSCLC cells, while pre-treatment with a miR-7 inhibitor blocked the down-regulation of Bax/Bcl-2. These results reveal that miR-7 is likely to be involved in breviscapine-induced growth suppression and apoptosis of NSCLC cells.

Scutellarin inhibited growth and induced apoptosis of tongue squamous cancer cells and their xenograft tumors in nude mice (Li, Huang, et al., 2013). These effects were associated with an altered expression of MMP-2 and 9, and integrin $\alpha_v\beta_6$ at mRNA and protein levels, as well as the expression of collagen fibers in the tumor microenvironment, possibly through the regulation of expression of transcription factor AP-1. In a HepG2 xenograft tumor model, treatment with scutellarin at 50 mg/kg per day for 35 days significantly inhibited the lung and the intrahepatic metastasis of HCC tumors (Ke et al., 2017). Scutellarin also reduced HepG2 cell viability and inhibited the migration and invasion of HCC cells in vitro. Scutellarin treatment significantly reduced STAT3 and Girders of actin filaments (Girdin) expression, as well as the STAT3 and Akt (protein kinase B or PKB) phosphorylation in HCC cells. STAT3 overexpression restored scutellarin-downregulated Girdin expression, Akt activation, migration and invasion of HCC cells. Overexpression of Girdin completely abrogated inhibition by scutellarin of the Akt phosphorylation and the migration and invasion of HCC cells. Therefore, scutellarin inhibits HCC cell metastasis in vivo and HCC migration and invasion in vitro by down-regulating the STAT3/Girdin/Akt signaling pathway. Scutellarin diminished the proliferation of B-lymphoma Namalwa cells in vitro and inhibited lymphoma growth in Namalwa cell-xenotransplanted mice without obvious toxicity (Feng et al., 2012). A mechanistic study showed that scutellarin at doses of <10 μM induced cell cycle arrest at the $G_0/G_1$ transition without inducing apoptosis, which was accompanied by the down-regulation of cyclin D1 and CDK4 expression. But at concentrations of 15 μM or above, scutellarin promoted Namalwa cell apoptosis, which was partially associated with activation of caspases (Feng et al., 2012).

### 5.9. Anti-neurodegeneration

Scutellarin is known to protect against the Aβ-induced impairment of learning ability and memory in rats. In this model, scutellarin reduced some of the deleterious effects of Aβ, possibly by stimulating the nicotinic acetylcholine receptor or nAChR protein translation and by regulating cholinesterase activity (Guo, Guan, and Wang, 2011). In a separate study using a similar model, animals treated with Aβ exhibited impaired learning and memory, reduced SOD activity, elevated monoamine oxidase activity, increased protein levels of IL-1β, IL-6, and TNF-α, and higher percentage of apoptotic neurons in the brain (Guo et al., 2013). Interestingly, all of these effects were ameliorated by administration of scutellarin. These findings indicate that the deficits in learning and memory observed in rats receiving Aβ are in part due to elevated oxidative stress and inflammation, which result in apoptosis, and scutellarin may prevent these deleterious effects. When applied to cultured neuronal cells, Aβ may cause damage to the cells and induce cell death, whereas scutellarin inhibits the aggregation of Aβ in vitro and prevented cell death in neuronal cells exposed to Aβ (Zhu et al., 2009).

### 5.10. Anti-diabetic effect

Radix Scutellariae, which is the root of *Scutellaria bacicalensis*, has been used for the improvement of blood glucose homeostasis. Scutellarin and baicalin are two bioactive components of Radix Scutellariae (Tahtah et al., 2015). Baicalin, which differs from scutellarin structurally by lacking the hydroxyl group at position C-4′, has been shown to ameliorate the metabolic disorders and hepatic steatosis in rats fed a high-fat diet, when given for a long period of time (Guo et al., 2009). The effects of scutellarin and baicalin on glucose homeostasis were compared (Yang et al., 2017). Both scutellarin and baicalin promoted glucose disposal in mice and in adipocytes with different mechanisms. Baicalin promoted glucose uptake in adipocytes by regulating the activation of AMP-activated protein kinase (AMPK), whereas scutellarin acted in a manner that is dependent on Akt activation. Moreover, scutellarin and baicalin in combination synergistically enhanced glucose uptake in adipocytes. Therefore, baicalin and scutellarin, though structurally similar, promoted glucose disposal in adipocytes by differentially regulating AMPK and Akt activities.

Breviscapine protected against diabetic cardiomyopathy in rats by inhibiting the expression of PKC and phospholamban, and by increasing the expression of protein phosphatase inhibitor-1, $Ca^{2+}$-ATPase, and the ryanodine receptor (Wang, Zhang, Zhu, Fu, and Zhou, 2010). Breviscapine at 65 μmol/L and 108 μmol/L attenuated high glucose-stimulated proliferation and migration of vascular smooth muscle cells (He, Xue, Li, and Zhou, 2012). Mechanistically, exposure of vascular smooth muscle cells to a high glucose medium activated PKC-β2 and ERK1/2 MAPK, but not p38 and JNK MAPK. Pretreatment with breviscapine blocked the high glucose-induced increase of the ERK1/2 activity, but not that of the PKC-β2 activity. Therefore, breviscapine ameliorates high glucose-induced proliferation and migration of vascular smooth muscle cells by inhibiting the ERK1/2 MAPK signaling. In a separate study, scutellarin inhibited hypoxia and moderately high glucose-induced proliferation of human retinal endothelial cells, which is likely to be mediated through the inhibition of VEGF expression by scutellarin (Gao, Zhu, Tang, Wang, and Ren, 2008).

### 5.11. Inhibition and molecular docking of Helicobacter pylori urease

Scutellarin and baicalin are two major components of *Scutellaria baicalenssi* Georgi that has been used as a "heat-clearing and detoxification" agent in traditional Chinese medicine for the treatment of *Helicobacter pylori*-related gastrointestinal disorders. *Helicobacter pylori* urease is a major pathogenic factor for *Helicobacter* infection. The urease is a nickel-dependent amidohydrolase that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Pivotal catalytic characteristics common to ureases are the nickel ions ($Ni^{2+}$) and the sulfhydryl groups within or near the active site of the enzyme, which provide a number of ways for with which they can interact with urease inhibitors.

Scutellarin and baicalin were shown to inhibit *Helicobacter pylori* urease in a dose- and time-independent manner, with $IC_{50}$ of $0.47 \pm 0.04$ mM and $0.82 \pm 0.07$ mM, respectively, compared to acetohydroxamic acid, a known inhibitor of the urease with an $IC_{50}$ of $0.14 \pm 0.05$ mM (Yu et al., 2015). A structure-activity analysis revealed that the 4′-hydroxyl group of scutellarin gave the flavones an advantage to binding *Helicobacter pylori* urease. Inhibition was non-competitive and reversible with inhibition constants ($K_i$) of $0.18 \pm 0.02$ mM and $0.14 \pm 0.01$ mM for scutellarin and baicalin, respectively. Inhibition involved the blockage of the SH groups of the urease, because thiol reagents, such as L,D-dithiothreitol, L-cysteine and glutathione, abolished the inhibitory action. Molecular docking supported the structure-activity analysis and revealed that scutellarin and baicalin interacted with Cys321 on the mobile flap through an S-H.pi interaction around the active site of the urease. These findings suggest that scutellarin and baicalin are effective urease inhibitors for the treatment of *Helicobacter pylori* infection.

## 6. Toxicity

Scutellarin and breviscapine are minimally toxic or nontoxic in animals. It was found that the maximal tolerated dose of scutellarin was >10 g/kg in mice and, as such, its acute lethal dose ($LD_{50}$) could not be

determined experimentally (Li et al., 2011). In a subacute study, an oral administration of scutellarin at a dose of 100 or 500 mg/kg daily for up to 30 days did not result in death or significant changes in hematology, blood chemistry, and urinalysis, except a non-dose related decrease in the levels of BUN and triglyceride. Moreover, scutellarin is unlikely to cause clinically significant drug-drug interactions in humans, when co-administered with the substrates of six cytochrome P450 enzymes, i.e., CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, and P-glycoprotein (P-gp, multidrug resistance protein or MDR); inhibition of these CYPs and P-gp are considered as the most frequent and clinically important pharmacokinetic causes among the various possible factors for drug-drug interactions (Han et al., 2014).

The $LD_{50}$ values of breviscapine (dengzhanxixin injection) by i.v. for female and male rodents were 1676.75 and 1740.76 mg/kg, respectively, whereas the $LD_{50}$ by i.p. was 1770.92 mg/kg; these values are equivalent to >250 times the maximum daily dosage, i.e., 6 mg/kg, used in clinical applications (Li, Lin, et al., 2015). A long-term toxicity study revealed that dengzhanxixin injection given to rats once daily by i.p. for 2 months is not toxic at a dose of 120 mg/kg, which is 20 times higher than the maximal daily dose of 6 mg/kg used clinically; but Beagle dogs receiving dengzhanxixin at 160 mg/kg by i.v. infusion for 60 days had elevated serum creatinine (Li, Lin, et al., 2015). These animal studies support the conclusion that both scutellarin and breviscapine have a sufficient margin of safety for therapeutic use.

The clinical safety of scutellarin and breviscapine was analyzed in a number of large scale studies. In a prospective, registry-based, post-marketing clinical safety monitoring study of dengzhanxixin injection, 15,962 cases were enrolled in the monitoring by November 2013 and 16 adverse drug reactions (ADR)/adverse drug events (ADE) were recorded. The ADR/ADE rate was 0.1002%. The ADR symptoms observed include rash (16.00%), chills (16.00%, and fever (16.00%), which have been observed for other plant-derived injections. A multi-center, randomized and controlled trial of dengzhanxixin injection was conducted for the treatment of acute ischemic stroke from June 2008 to June 2010. A total of 343 patients received dengzhanxixin injection plus basic therapy with Western medicine and the recovery techniques of traditional Chinese medicine, whereas the control group (335 cases) received Western medicine only (Li, Lin, et al., 2015). Clinical safety evaluation revealed 11 cases of ADR/ADE, of which four cases were associated with dengzhanxixin injection, with an incidence of ADRs being 1.17%, i.e., 4/343. The symptoms include fever, chills, rashes, nausea, dizziness, and palpitation. It was concluded that there is significant evidence that dengzhanxixin injection is safe and effective in clinical use for cerebrovascular and cardiovascular diseases.

## 7. New strategies to improve pharmacokinetics and efficacy

### 7.1. Drug complexes with improved drug delivery

#### 7.1.1. Nanoparticle carrier with amphiphilic derivatives

Scutellarin has been shown to be effective in treating diabetic vascular endothelial cell dysfunction, but its clinical application is limited by its low oral bioavailability. A novel intestine-targeted nanoparticle carrier with amphiphilic chitosan derivatives loaded with scutellarin (Chit-DC-VB12-Scu) was created to enhance scutellarin's bioavailability for its therapeutic effect in experimental diabetic retinopathy (Wang et al., 2017). The bioavailability study in rats revealed that Chit-DC-VB12-Scu had an AUC that was two to three-fold higher than that of free scutellarin. Moreover, Chit-DC-VB12-Scu alleviated the structural disorder of intraretinal neovessels, reduced the central retinal artery resistivity index, and inhibited the retinal neovascularization by down-regulating the expression of angiogenesis proteins, such as VEGF, VEGFR2, and vWF, in the retina of diabetic rats.

#### 7.1.2. Lipid emulsion and cyclic oligosaccharides

Lipid emulsions can be used as a vehicle for intravenous delivery of medications. A series of monooleate-modified polyethylene glycol (PEG) with the active carboxylic terminus on the other end (MO-PEG-COOH) to modify the lipid emulsion surface were used to obtain sterically stabilized lipid emulsions for the delivery of breviscapine (Xiong, Xiong, Yao, Chen, and Gu, 2011). It was concluded that an increased plasma concentration of breviscapine by surface modification of the lipid emulsions can enhance the pharmacological activity of breviscapine in promoting blood circulation. In another study, breviscapine was complexed with β-cyclodextrin, a cyclic oligosaccharide complex, to improve its intestinal absorption. Compared with breviscapine, the breviscapine-β-cyclodextrin complex had a significantly better absorption rate than breviscapine alone in the rat intestine (Zhang, Ping, Guo, and Cao, 2005).

### 7.2. Drug derivatives with improved pharmacokinetics and efficacy

#### 7.2.1. PEGylation for cerebral penetration

The polymer PEG or methyl PEG (m-PEG) can be attached to therapeutics to improve their clinical safety and efficacy, a process called PEGylation. PEGylation has been used to extend the body retention time, enhance the distribution into tissues, decrease the adverse effects, and increase the resistance to degradation of many drugs, which generally improves patient compliance. In one study, a number of PEG-scutellarin prodrugs were synthesized by modifying the carboxyl and phenolic hydroxyl groups of scutellarin with m-PEG of different molecular weights (400–3000) (Lu et al., 2010). The water solubility of the prodrugs increased remarkably and reached a maximum value of 783.88 mg/mL, whereas that of scutellarin is merely 0.02 mg/mL. The anti-infarction effects of four PEG prodrugs with a high water solubility were evaluated in a cerebral I/R MCAO model. The prodrug 7e with an m-PEG (molecular weight = 2000) attached at the carboxyl group (Fig. 4a) was shown to significantly reduce the infarct area from 27.2% to 12.2% (33.3% for the control) and decrease the neurologic deficit score from 2.77 to 1.32 (2.85 for the control). The half-life of the prodrug 7e was significantly longer than that of scutellarin (18.62 min versus 3.03 min). Importantly, the prodrug 7e had a 2.1-fold higher tissue distribution in the brain than scutellarin, indicating that PEGylation increased the BBB penetration of scutellarin and may thereby provide more effective protection against cerebral I/R injury than scutellarin alone (Lu et al., 2012).

#### 7.2.2. Mannich bases as thrombin inhibitors

Thrombin is a multifunctional serine protease that not only catalyzes the proteolytic cleavage of fibrinogen into insoluble fibrin leading to clot formation, but also serves as a potent platelet agonist and amplifies its own generation. Therefore, thrombin represents a key therapeutic target in treating ischemic cerebral disease. Molecular docking of thrombin (2R2M) with scutellarein reveals that the B ring and C ring of scutellarein could interact well with the S1 and S2 pockets of thrombin, respectively (Li et al., 2012). The A ring partly interacts with the S3 pocket of thrombin, suggesting that the inhibitory activity could be improved if a side chain is introduced at position 8 in the A ring. Accordingly, two series of 8-aminomethylated derivatives were prepared via a Mannich reaction of scutellarein with aliphatic amines, alicyclic amines, and formaldehyde, which involves the nucleophilic addition of the amine to a carbonyl group followed by dehydration to the Schiff base. It was found that the morpholinyl aminomethylene substituent derivative (3d) (Fig. 4b) had a stronger anticoagulant activity, better water solubility, and higher antioxidant activity, than scutellarein, and thus warrants further development as an anti-ischemic stroke agent.

#### 7.2.3. Glucose-containing derivatives as neuroprotective agents

As a flavonoid glucuronide, scutellarin is readily converted to its aglycone by β-glucuronidase of the intestinal microflora, which in part accounts for the very low plasma concentration of scutellarin after an

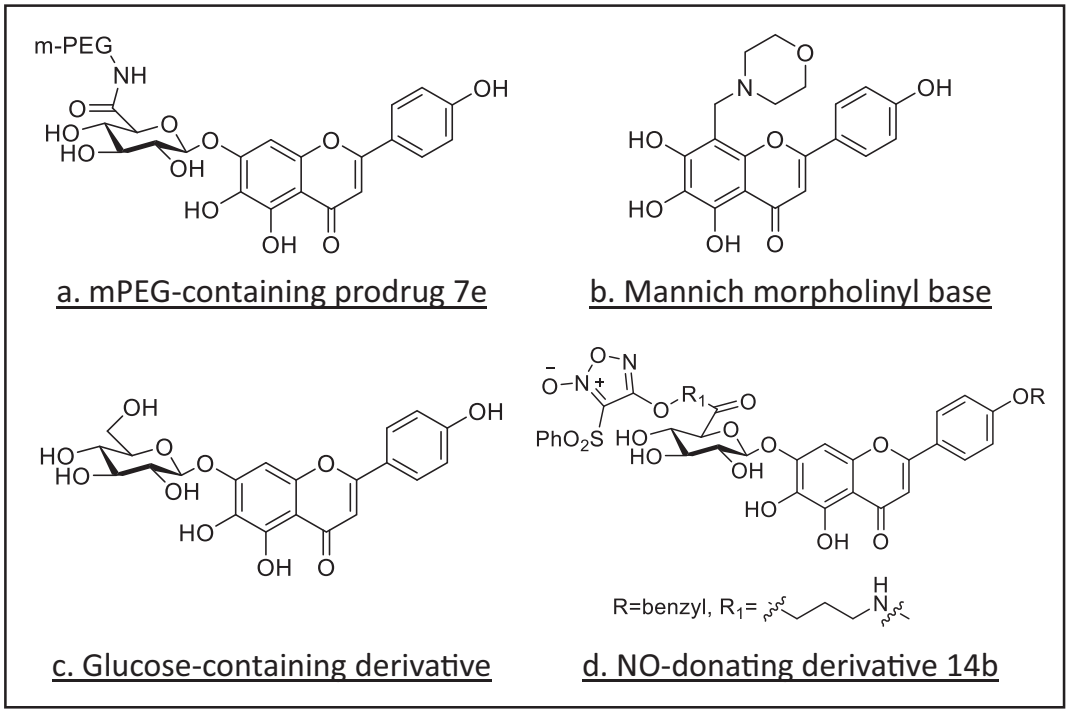


**Fig. 4.** Scutellarin derivatives with enhanced pharmacokinetic property and efficacy. The structures of representative scutellarin derivatives with improved pharmacokinetics and/or enhanced drug efficacy but low toxicity are shown.

oral administration. In one study, a glucose moiety was introduced at the C-7 position of scutellarein via a glucosidic bond (Fig. 4c), which avoids β-glucuronide cleavage by β-glucuronidase (Li et al., 2012). This glucose-containing scutellarein derivative exhibited potent radical scavenging activities in vitro. The water solubility and the anticoagulant and neuroprotective activities of the glucose-containing derivative were also significantly higher than those of scutellarin.

*7.2.4. NO-donating derivatives as apoptosis inducers*

NO at a high concentration could induce apoptosis and inhibit metastasis of tumor cells, or sensitize tumor cells to chemotherapy, radiation, and immunotherapy. NO donors, such as furoxans and nitrates, can be added to drug molecules to enhance their anticancer efficacy. A series of NO-donating scutellarin derivatives (14–17) were synthesized to explore new scutellarin-based drugs with high efficiency and low toxicity as anticancer agents (Han et al., 2017). Compound 14b (Fig. 4d) had the strongest activity with $IC_{50}$ values of 2.96, 7.25, 0.09 and 0.50 μM against tumor cells MCF-7, HCT-116, PC-3, and HepG2, respectively; at the same time, it displayed a low toxicity against normal human liver L-O2 cells with an $IC_{50}$ of 47.96 μM, showing a differential selectivity between normal and malignant liver cells. Mechanistically, compounds 14b and 15a could induce apoptosis and cell cycle arrest at the S phase and cause mitochondrial dysfunctions in the HepG2 and PC-3 cell lines, respectively. Furthermore, 14b could induce apoptosis by down-regulating the levels of procaspase-3 and by inhibiting the expression of survivin, c-IAP1, HSP27, HSP60, HSP70, HO-1/HMOX1/HSP32, and HO-2/HMOX2 in HepG2 cell line. These results suggest that compound 14b is a promising drug candidate against liver cancer.

## 8. Conclusion

The development of therapeutic agents from traditional herbal medicines has become a promising direction for the treatment of a range of diseases and pathological conditions, such as cerebral and myocardial ischemia, diabetic complications, neurodegeneration, and cancer. In part, this is because the pathological development of these illnesses is complex, involving the dynamic interactions among multiple functions and structures that are not well understood. This complexity is often well beyond that which a single drug that generally targets a single or a few molecules can cope. Moreover, current medications for these diseases are known to cause serious adverse effects in certain patient populations. Mechanistically, these adverse effects can be the same as or different from their therapeutic effects, thus often referred to as "on-target" or "off-target" side effects, respectively (Ma and Lu, 2011). In the case of ischemia, the timely blood reperfusion by means of thrombolytic therapy or surgical intervention is the treatment of choice, as it would effectively relieve tissue ischemia. However, prolonged thrombolysis may cause bleeding, whereas reperfusion itself may stimulate the production of ROS and RNS, activate endothelial cells, and boost inflammatory infiltration and proinflammatory cytokine secretion; this would exacerbate the functional derangement and tissue damage to cause the so-called "I/R" injury. On the other hand, herbal medications that often have a low toxicity profile and low cost may exert multiple therapeutic effects, including cellular protection, anti-inflammation, anti-oxidative stress, and neural and cardiovascular protective effects and, therefore, are being increasingly used in combination with conventional medicine to treat these diseases. Scutellarin and breviscapine are examples of medications developed from traditional Chinese herbal medicine with demonstrated therapeutic effects and clinical benefits for ischemic cerebral and cardiovascular diseases and diabetic complications. Moreover, recent advances in understanding the pharmacology and pharmacokinetics of scutellarin have led to new strategies for drug development.

The multi-effect nature of many herbal medicines may derive from their mixed herbal components, which often consist of hundreds of compounds that exert their effects on disease by binding multiple targets to result in complex and synergistic therapeutic activities. Alternatively, some herbal compounds may exhibit multiple intrinsic activities even as monomers, owing to their unique structures and pharmacologic actions. In either scenario, it is generally very challenging to identify the key bioactive components and their molecular targets of an herbal medicine. Development of extract preparations from herbal medicine is perhaps one solution to this issue, as an extract would replicate the total therapeutic effects of an herbal medicine and, at the same time, may

facilitate the identification of the bioactive ingredients and mechanisms of action through pharmacological studies of the extract preparation. The widely used breviscapine preparations and the identification of scutellarin as the active monomer component of breviscapine demonstrate the effectiveness of this extract-to-monomer approach in the development of anti-ischemic drugs. Scutellarin itself exerts multiple pharmacological activities and therapeutic effects, which may reflect its structure as a flavonoid glucuronide. Other plant-derived flavonoid glucuronides, such as baicalin, have also been shown to possess multiple functions, including anxiolytic and anti-cancer effects (Xu et al., 2006). These findings suggest that flavonoid glucuronides as a group may have unique pharmacological values.

Although scatellarin and breviscapine have been used clinically to treat a range of diseases and have been extensively studied pharmacologically in laboratory animals, there are limitations to be considered when evaluating and drawing conclusions from these studies. For instance, many clinical studies on scutellarin and breviscapine were performed with low methodological quality, including the small size of patient populations, a lack of double blinded and randomized designs, and insufficient controls. While meta-analyses of combined studies have been used to improve the validity of these investigations, large scale, randomized, prospective, and multi-centered studies are still much in need for the evaluation of the efficacy and safety of the drugs in human populations.

Additionally, several other issues are commonly seen in clinical studies of scutellarin. For instance, the doses of extract preparations given to patients are often expressed in volumes such as milliliter, which makes it difficult, if not all impossible, to compare clinical effects between different preparations. In a combination therapy with scutellarin and a conventional or Western medicine-based therapy, all treatments should be clearly indicated and their effects clearly distinguished as to which treatment is the major driver of the therapeutic effect and which has merely a synergistic effect. Last but not least, the long-term effect of scutellarin treatment should be measured in clinical studies, such as the re-occurrence rate, mortality, and long-term disability rate after a treatment.

For experimental and laboratory-based research on scutellarin, combined studies of human and animal, in vivo and in vitro, and mechanistic and molecular investigations are necessary, in order to provide a comprehensive analysis of the drug effects that is on par with the standards and common practice of pharmacological research in the world. Notably, a few animal systems, such as the MCAO rat model for cerebral ischemia and the LAD closure for myocardial infarction, have been well utilized for the analysis of the therapeutic effect of scutellarin on I/R injury in vivo in a quantitative and mechanistic manner. Nevertheless, many studies have been performed to characterize the pharmacological effects of scutellarin with very limited insights into its mechanism of action at the level of drug targets and molecular pathways.

Scutellarin has been shown to exhibit unique pharmacokinetic behaviors in humans and animals. How these findings translate into the pharmacological activities of scutellarin remains unclear. For instance, what constitutes the chemical form(s) that mediate the therapeutic effects of scutellarin and breviscapine in target tissues, such as the CNS, has not been well addressed. The serum level of scutellarin after an oral exposure is exceptionally low in humans, in comparison with those of scutellarein and its mono and di-glucuronide conjugates. Moreover, scutellarein, but not the glucuronide conjugates, readily crosses the plasma membrane. Therefore, it was postulated that a metabolite (s), but not scutellarin itself, mediates the pharmacological effects in target organs. Identification of this active form would help identify the drug target, delineate the dose-effect relationship, and facilitate future drug development from scutellarin.

Currently, a majority of the clinical therapeutic effects of scutellarin are observed with breviscapine, whereas the scutellarin compound and its derivatives are mainly used for pharmacological, pharmacokinetic, and exploratory studies in animals and in vitro models. This lack of clinical use of scutellarin itself in part reflects the low bioavailability and unfavorable pharmacokinetics of the compound in humans, which provides both a challenge and an opportunity for translational research on scutellarin for drug development and clinical application in future studies.

In aggregate, despite the uncertainties and shortcomings discussed above, the research on scutellarin has been progressing rapidly and one may predict that scutellarin and its related medications would gain even more attention in the treatment of ischemic injury and certain chronic diseases in the coming years.

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## Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

## Conflict of interest statement

The authors declare that there are no conflicts of interest.

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