Traditional Chinese Medicine on Cholesterol-Dependent Vascular Smooth Muscle Abnormal Contraction

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INTRODUCTION

Cardiovascular and cerebrovascular diseases, including cardiac and cerebral infarction resulting from blood flow decreases or stops caused by vascular smooth muscle abnormal contraction, have been considered as one of the leading causes of morbidity and mortality in all of world. Numerous evidences have indicated that abnormal lipoprotein metabolism, hypercholesterolemia, is a key risk factor for cardiovascular and cerebrovascular diseases [1–5]. The elevated level of LDL-cholesterol (LDL-c) is linked to an increased risk of cardiovascular and cerebrovascular events [6–8]. During the past two decades, sphingosylphosphorylcholine (SPC) as a spasmogen has been demonstrated to trigger the vascular smooth muscle abnormal contraction [9–11]. This abnormal contraction is dependent on the levels of serum cholesterol and LDL-c [12]. Therefore, lowering serum cholesterol levels may prevent cardiovascular disease caused by vascular smooth muscle abnormal contraction.

ABSTRACT

Vascular smooth muscle abnormal contraction is the Ca\(^{2+}\)-independent, named as the Ca\(^{2+}\)-sensitization contraction. Sphingosylphosphorylcholine (SPC) is a causal factor of vascular abnormal contraction and triggers this contraction by the Fyn/Rho-kinase signaling pathway. The SPC-induced abnormal contraction is dependent on the levels of total cholesterol and LDL-cholesterol. Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) effectively inhibit the SPC-induced contraction. Recently, we have screened a series of active monomers from traditional Chinese medicine (TCM) and discovered some ones have potential inhibitory effects on the SPC-induced abnormal contraction. In this review, we summarize the progress of recent research on vascular smooth muscle abnormal contraction. We also provide the new findings of active monomers from TCM action on vascular smooth muscle abnormal contraction.

Keywords: Traditional Chinese medicine, sphingosylphosphorylcholine, vascular abnormal contraction, cholesterol, flavone

Polyunsaturated fatty acids (PUFAs) have two families of omega-3 (n-3) and omega-6 (n-6) PUFAs. n-3 PUFAs have lowering-cholesterol effect and are thought to have beneficial effect on prevention of cardiovascular diseases, while n-6 PUFAs are considered proinflammatory and thought to negatively affect the cardio-vasculature. Two n-3 PUFAs, eicosapentaenoic acid (EPA, 20:5 n-3) and docosapentaenoic acid (DPA, 22:5 n-3), effectively inhibit the SPC-induced vascular smooth muscle abnormal contraction [13,14]. Another form of DPA, n-6 DPA, inhibits the SPC-induced abnormal vascular contraction and has the same strong effect as n-3 DPA [13]. Although EPA and DPA have potential effects on the SPC-induced vascular abnormal contraction, they could not be intravenous use in the condition of emergent for their poor solubility in water.

Traditional Chinese medicine (TCM) has been used for 2000 years with high safety. Most TCMs generally originate from some natural plant products including dietary sources or herbal medicines. TCM has been demonstrated to be effective in control of cardiovascular risk factors, such as hypercholesterolemia, hypertension, and diabetes [15–18]. Many basic and clinical studies have shown that TCM are widely used to treat cardiovascular diseases as one of many alternatives [19–24]. Add-on therapy with TCM is considered to be an efficacious approach and has been used to prevent and treat cardiovascular diseases [25–27]. In this review, we will provide recent research progress that the active

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monomers from TCM on vascular smooth muscle abnormal contraction. We also elaborate current understanding on vascular smooth muscle abnormal contraction and the role of cholesterol in this contraction.

**VASCULAR SMOOTH MUSCLE ABNORMAL CONTRACTION**

**Vascular smooth muscle abnormal contraction is mediated by the SPC-Fyn-Rho-kinase (ROK) pathway**

There are two types of contractions, the Ca$^{2+}$-dependent contraction and the Ca$^{2+}$-independent contraction, in vascular smooth muscle. The former is induced by an increase of Ca$^{2+}$ concentration and plays an important role in the maintenance of physiologic blood pressure [28,29], known as vascular normal contraction; the latter is not dependent in Ca$^{2+}$ concentration and induces vasospasm via ROK, known as vascular abnormal contraction, has been proposed a major cause of cardio-cerebral-vascular diseases, such as coronary artery vasospasm and cerebral vasospasm [30,31]. The ideal treatment for vascular smooth muscle abnormal contraction is to identify a substance which effectively inhibits vascular abnormal contraction with little or no effect on vascular normal contraction.

The molecular mechanism underlying the vascular smooth muscle abnormal contraction has not been completely elucidated, but a large number of evidences have indicated that ROK-mediated Ca$^{2+}$-independent contraction of vascular smooth muscle is associated with coronary artery and cerebral abnormal contraction [30–32]. As an upstream signaling molecule of ROK, SPC is identified to induce vascular smooth muscle abnormal contraction in porcine coronary arterial strips by activating ROK [9,10]. The specific ROK inhibitor Y27632 inhibited the SPC-induced contraction [10], suggesting that ROK is involved in the SPC-induced vascular abnormal contraction. Dominant negative ROK (dn-ROK) abolished the SPC-induced abnormal contraction in vascular smooth muscle strips permeabilized with β-escin [10]. In addition, SPC induced the translocation of ROK from the cytosol to the cell membrane of vascular smooth muscle cells [10]. These results indicate that the ROK as a downstream molecule of SPC mediates vascular smooth muscle abnormal contraction.

The following study demonstrated that Fyn tyrosine kinase, a member of the Src family protein tyrosine kinases, is involved in the SPC-ROK pathway mediated abnormal contraction as an upstream signaling molecule of ROK. PP1, a specific inhibitor of Fyn, abolished the SPC-induced vascular abnormal contraction [33]. In contrast, PP3, an inactive analogue of PP1, could not inhibit the SPC-induced abnormal contraction, suggesting that Fyn is involved in the SPC-induced abnormal contraction. In addition, SPC induced translocation of Fyn from the cytosol to the cell membrane [33]. The specific inhibitors for S-palmitoylation of Fyn, 2-bromopalmitate and EPA, inhibited the SPC-induced abnormal contraction and translocation of Fyn [33]. Moreover, PP1 and EPA as Fyn inhibitors subsequently inhibit translocation and activation of ROK, resulting in inhibition of vascular abnormal contraction. These results indicate that Fyn as an upstream signaling molecule of ROK is involved in the SPC-ROK signaling pathway mediated vascular smooth muscle abnormal contraction leading to vasospasm.

**Vascular smooth muscle abnormal contraction is dependent on cholesterol level**

Cholesterol is a vital component in the cell membrane, where it helps to maintain the integrity of cell membrane, and plays a role in facilitating cell signaling [34,35]. Cholesterol is not soluble in the blood and is carried into the blood by the two main types of lipoproteins: LDL, also known as “bad cholesterol”, and HDL, also known as “good cholesterol”.

To clarify the relationship between serum cholesterol level and vascular abnormal contraction, the possible cholesterol dependence of the SPC-induced abnormal contraction was investigated in humans and rabbits with hypercholesterolemia [12]. In arterial strips from hypercholesterolemic patients, SPC strongly induced abnormal contraction of vascular smooth muscle. In contrast, this contraction was not observed in arteries obtained from patients with normal serum cholesterol levels, indicating that the SPC-induced vascular abnormal contraction is related to the level of serum cholesterol. Further studies showed that the SPC-induced vascular abnormal contraction was well correlated with the amount of serum total cholesterol and LDL-c [12]. Clinical trials also showed that SPC-induced contractions were significantly decreased for hypercholesterolemic patients treated with cholesterol-lowering drugs, and the decrease is correlated with the levels of serum total cholesterol and LDL-c [12]. On the other hand, an inverse correlation was found between the extent of the SPC-induced contraction and the ratio of HDL-c level to the total cholesterol level, indicating protective role of HDL-c on cardiovascular diseases [12]. In addition, the specific depletion of cholesterol in vascular tissue by β-cyclodextrin (β-CD) also inhibits the SPC-induced vascular abnormal contraction [12]. These findings suggest that cholesterol directly and specifically primes vascular smooth muscle to induce the vascular abnormal contraction mediated by the SPC-ROK pathway [12]. Recently, our results further have confirmed that the SPC-induced vascular abnormal contraction is also correlated with the levels of cholesterol in the vascular smooth muscle tissue. In the range of 13.72–40.66 mg cholesterol/10 mg tissues, the SPC-induced contraction was correlated with the amount of cholesterol in the tissue (unpublished data). This observation further reveals the cholesterol dependence of the SPC-induced vascular abnormal contraction. The signaling pathway of the SPC-induced vascular abnormal contraction and the role of cholesterol in this contraction are shown as Fig. 1.
INHIBITORY EFFECTS OF PUFAS ON VASCULAR SMOOTH MUSCLE ABNORMAL CONTRACTION

Inhibitory effect of EPA on vascular smooth muscle abnormal contraction

EPA, one of n-3 PUFAs, has been found to play an important role in lowering cholesterol level and prevention of cardiovascular diseases [11,36,37]. EPA inhibited the SPC-induced vascular abnormal contraction via inhibiting Fyn translocation from the cytosol to the cell membrane [33]. Our previous study demonstrated that the oral administration of EPA to rabbits lowered the SPC-induced vascular abnormal contraction. Simultaneously we found that treatment with EPA reduced the SPC-induced vascular abnormal contraction in hypercholesterolemic patients [12]. Recently, Shirao et al. reported EPA strongly inhibits the SPC-induced vasoconstriction and subarachnoid hemorrhage-induced cerebral vasospasm in dogs [11]. These observations suggest that inhibition of the SPC-induced abnormal contraction by EPA is associated with lowering total cholesterol levels in serum, and cholesterol-lowering therapy may inhibit SPC-induced abnormal vascular contraction.

Inhibitory effect of DPA on vascular smooth muscle abnormal contraction

Unlike EPA, DPA has two forms, n-3 DPA and n-6 DPA. The n-3 and n-6 DPA are reported to be beneficial in improving the lipoprotein profile and aortic function in hamsters fed a high cholesterol diet [38]. Rissanen et al. also reported that a high proportion of fish-derived DHA and DPA in serum is associated with a decreased risk of acute coronary syndrome [39]. We found that both n-3 DPA and n-6 DPA inhibit the SPC-induced vascular abnormal contraction with little effect on the high K⁺ depolarization-induced vascular normal contraction [13]. Surprisingly, n-6 DPA inhibited the SPC-induced abnormal contraction to the same degree as that observed with a treatment of n-3 DPA, suggesting that including n-6 PUFAs in the diet may be beneficial for the prevention of vascular abnormal contraction. The mechanism that n-3 and n-6 DPA effectively inhibit SPC-induced contraction by inhibiting ROK activation and its translocation to the cell membrane was clarified [13]. Further study is needed to verify the correlation between cholesterol-lowering effect and the inhibitory effect of the SPC-induced vascular abnormal contraction.

EFFECTS OF ACTIVE MONOMERS FROM TCM ON VASCULAR SMOOTH MUSCLE ABNORMAL CONTRACTION

Chinese herbal medicine, with thousands of years history, attract the attention of researchers around the world because Chinese herbal medicines most are plants and are often perceived as natural. There is an increasing interest in TCM to treat all kinds of diseases and conditions including cardiovascular diseases caused by hypercholesterolemia [37,40,41]. To find a substance having a potent inhibitory effect on vascular abnormal contraction simultaneously with no or little effects on vascular normal contraction, we focused on some active monomers from traditional Chinese medicines. After screening for a large
number of monomers, we found that flavones contained in TCM have strongly inhibitory effects on the SPC-induced vascular abnormal contraction. The chemical structures of active monomers form TCM are shown as in Fig. 2. In the following sections, we will focus on the progress made by some of the most promising monomers including luteolin and baicalein. We also provide some preliminary results on the effects of two monomers to the SPC-induced vascular smooth muscle abnormal contraction.

**Luteolin**

Luteolin is a common flavonoid that exists in many types of plants including fruits and vegetables. Luteolin is also isolated from Chinese herbal medicines such as Ban Zhi Lian [42], Ju Hua (Flos Chrysanthemi) [43] and Bai Mao Xia Ku Cao (Decumbent Bugle Herb) [44] (Table 2). The chemical structure of luteolin is shown as in Fig. 2.

Earlier experimental data have shown that luteolin possesses potent anti-oxidant, anti-inflammatory, anti-microbial, and anti-cancer activities [45]. Recently, there has been an increase in a number of reports on the cardiovascular effects of luteolin [46–48]. Emerging evidences have demonstrated that luteolin exerts multiple biological effects on the heart and vascular vessels including activating anti-apoptosis key protein Akt [49], attenuating oxidative stress [50], regulating MAPKs and NO synthases isozymes activities [51], and inhibiting proliferation and migration of vascular smooth muscle cells [52].

**Baicalein**

Baicalein is a flavone and an active ingredient in Scutellaria baicalensis Georgi or Scutellariae Radix [53,54], also called Huang Qin in China, the most widely used herb, which has been traditionally used as anti-inflammatory
and anti-cancer therapy. Baicalein is also isolated from Ban Zhi Lian [42]. The chemical structure of baicalein is shown as in Fig. 2.

Extensive studies showed the role of baicalein on treating and preventing various types of cancer, such as bladder cancer, breast cancer, cervical cancer, and so on [55]. The cardioprotective role of baicalein has been proved in several cell culture and small animal models. Lee et al. demonstrated that baicalein improves cardiac contractile function in endotoxaemic rats via induction of heme oxygenase-1 and suppression of inflammatory responses [56]. Baicalein was also reported to inhibit vascular smooth muscle cells proliferation and migration [57,58].

**Effects of luteolin and baicalein on vascular smooth muscle abnormal contraction**

To identify a substance which effectively inhibits vascular abnormal contraction with little or no effect on vascular normal contraction, a large number of compounds from TCM were screened. Until now, we found that two compounds belonging to flavone subfamily, luteolin and baicalein, potently inhibited the SPC-induced vascular abnormal contraction. In contrast, they showed little effects on the high K\(^+\) depolarization-induced normal contraction (Table 1), suggesting that they may be promising candidates to reduce the occurrence of cardiovascular diseases resulting from vascular abnormal contraction. In addition, we also investigated other compounds structurally similar to flavone subfamily, such as flavonol, flavanone, and flavanol subfamilies (Fig. 2 and Table 1). Quercetin from flavonol subfamily had a little inhibitory effect on the SPC-induced abnormal contraction. Naringenin and hesperetin from flavone subfamily showed little inhibitory effects on the SPC-induced abnormal contraction but strongly inhibitory effects on the high K\(^+\) depolarization-induced normal contraction. Epicatechin and catechin from flavanols showed little or no effects on either SPC-induced abnormal contraction or the high K\(^+\) depolarization-induced normal contraction. Based on these results, we speculate that including active flavones in TCM, such as luteolin and baicalein may be beneficial for the prevention of cardiovascular and cerebrovascular diseases arising from vascular abnormal contraction. To further confirm their effects on the SPC-induced vascular abnormal contraction, a series of related studies are underway.

**Effects of luteolin and baicalein on lowering cholesterol**

Lowering cholesterol is an effective approach to reduce the incident rate of cardiovascular and cerebrovascular diseases. Flavonoids have been demonstrated to decrease total cholesterol (TC), triglycerides (TG), LDL-\(c\) and increase HDL-cholesterol (HDL-\(c\)) in animal and human studies [59–61]. Nekohashi et al. compared the cholesterol levels between rats fed both cholesterol and luteolin and those fed only cholesterol, they found that luteolin significantly decreases the serum cholesterol levels [62]. Baicalein was reported to reduce the serum free fatty acid and TG levels and liver TG content [63]. The lowering cholesterol effects of two compounds (luteolin and baicalein) were summarized as shown in Table 2. Further studies are needed to confirm whether they inhibit the SPC-induced vascular abnormal contraction through lowering the cholesterol levels. The roles of two monomers in regulating SPC-induced vascular abnormal contraction and cholesterol metabolism indicate that they could be used for treatment of cardiovascular diseases.

<table>
<thead>
<tr>
<th>Flavonoids subfamily</th>
<th>Active monomers</th>
<th>SPC-induced vascular abnormal contraction</th>
<th>High K(^+) depolarization-induced vascular normal contraction</th>
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<tr>
<td>Flavones</td>
<td>Luteolin</td>
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<td>Baicalein</td>
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<tr>
<td>Flavonols</td>
<td>Flavonol</td>
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<td>Quercetin</td>
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<td>Flavanols</td>
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Table 1: The summary shows the effects of monomers from traditional Chinese medicines on the SPC-induced vascular abnormal contraction and the high K\(^+\) depolarization-induced vascular normal contraction. The inhibitory effects of monomers are shown as follows: +++ means 70%–100% inhibitory effect; ++ means 30%–69% inhibitory effect; + means 1%–29% inhibitory effect; - means no inhibitory effect.
CONCLUSIONS AND FUTURE PERSPECTIVES

Cardiovascular diseases represent one of the leading causes of death worldwide. Vascular abnormal contraction, named vasospasm including cardiovascular and cerebrovascular vasospasm, remains a significant source of morbidity and mortality in sudden death and in patients after subarachnoid hemorrhage. As a spasmogen, SPC is identified to induce vascular smooth muscle abnormal contraction leading to vasospasm. This abnormal contraction is mediated by the SPC-Fyn-ROK signaling pathway and dependent on the level of cholesterol. Lowering the levels of cholesterol contributes to reduce the occurrence of cardiovascular and cerebrovascular events associated with vascular smooth muscle abnormal contraction. EPA and DPA effectively inhibit SPC-induced vascular abnormal contraction. In particular, n-6 DPA potentially inhibits the SPC-induced vascular abnormal contraction, providing a new insight for the prevention of cardiovascular and cerebrovascular abnormal contraction by n-6 PUFAs.

Flavones from traditional Chinese herbal medicines including luteolin and baicalein have potential to inhibit the SPC-induced vascular abnormal contraction. Despite the promising results of preclinical studies on luteolin and baicalein from traditional Chinese herbal medicines have been demonstrated in vitro, additional research is required to examine their effects in vivo. In future, clinical studies are greatly required for the development of active monomers from TCM as promising drugs to treat cardiovascular and cerebrovascular diseases.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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