Effects of natural products on ischemic heart diseases and cardiovascular system

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Key Words myocardial infarction; herbal medicine; antioxidants; cardiovascular system; Salvia miltiorrhiza

Introduction

Ischemic heart diseases remain the leading cause of death in most developed countries as seen over the past quarter-century. Reducing the mortality rate and prevention of myocardial infarction (MI) are of utmost importance. The use of Western medicinal drugs for the treatment of hypertension, congestive heart failure and post MI are widely accepted. Although some Traditional Chinese Medicines have been used in mainland, Hong Kong, Taiwan of China in clinics and other Chinese communities worldwide for centuries, our understanding of the scientific principles of these herbal drugs is still far from satisfactory. As a result, their wide spread use in patients is limited throughout the world, especially in Western societies, although some of them could be used as complementary medicines in the treatment of Western medicines or tonics to maintain good health. The mechanisms of action and interaction, and the chemicals they contain remain undetermined in most Chinese traditional medicines, and need to be further elucidated using modern biological tools.

As the medicinal value of Chinese traditional medicine cannot be ignored, researchers are gradually becoming interested in identification of the active principles in the extracts with intensive follow-up study of their mechanisms of action. In this review, we mainly examine some natural products of Chinese herbal origin, and focus on elaborating the mechanisms of their beneficial effects on ischemic heart diseases, especially on myocardial infarction. In fact, there are a large number of natural products that have been confirmed to have such effects. Guided by publications indexed in Medline, we focus on herbal medicines (crude extracts and pure compounds) that have been deeply studied in recent ten years.

Cardioprotective Effects of Herbal Extracts After Myocardial Infarction

Infarct size and survived myocardium The infarct size is an important parameter to evaluate the severity of acute myocardial infarction (MI). Pretreatment with trilinolein¹ isolated from Panax pseudoginseng, or magnolol and honokiol¹²,¹³, main components purified from Magnolia officinalis at the dosages of 0.1 and 0.01 mg/kg could significantly reduce the infarct size in rat models.

When compared the effects of Salvia miltiorrhiza Bunge (SMB, DanShen) with ramipril, an angiotensin converting enzyme (ACE) inhibitor, in rats induced with MI, Ji et al.¹⁴ found that DanShen reduced the ratios of infarct size to the left ventricular size, total heart weight, left ventricular weight and right ventricular weight to body weight as effectively as ramipril. Similar effect could be seen in treatment with lithospermic acid B from
Precontracted rat aorta mainly through endothelium-dependent mechanism, and partially via endothelium-independent way. This relaxation produced by UR was long-lasting.

Gallotannin, a component of *Paeoniae radix*, procyanidins, an active principle in *Crataegus* extract, and ginsenosides from the protopanaxatriol group were also proved to have an endothelium-dependent vasodilator effect on isolated rat aorta.

In addition, magnolol could relax vascular smooth muscle by releasing NO and inhibiting calcium influx through voltage-gated calcium channels in rat thoracic aorta *in vitro*. As excessive NO production due to elevated expression of iNOS may produce cytotoxic effects to cells in the vascular wall, magnolol and honokiol also could weakly inhibit inducible NO synthase (iNOS) enzyme activity, but potent decrease iNOS induction in lipopolysaccharide (LPS)-activated macrophages.

Cheung *et al* found that *Ginkgo biloba* (Gb) extract selectively inhibited iNOS expression without affecting eNOS-mediated NO production *in vitro*. Such inhibition of iNOS induction could also be seen in treatment with wogonin, baicalein and baicalin, isolated from the dry roots of *Scutellaria baicalensis* Georgi, and the active stilbenes, isolated from the rhizome of *Rheum undulatum* L. But it was also demonstrated that baicalein and baicalin enhanced the phenylephrine-induced contraction through inhibiting production or/and release of endothelial nitric oxide in the isolated rat mesenteric artery rings.

The relationship between NO and natural products, and the role NO plays in the treatment of ischemic heart diseases with natural products still need to be further elucidated.

**Effects on vasorelaxation** Endogenous nitric oxide (NO), once named endothelium-derived relaxing factor (EDRF), which plays a key role in numerous physiological and pathological processes, is synthesized from L-arginine by a family of nitric oxide synthase (NOS) isoenzymes including endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). NO acts as a vasodilator when produced by constitutive NOS, which is composed of eNOS and nNOS in endothelial cells. Several natural products have vasorelaxant effects due to the release of NO. Cheung and co-workers recently verified that magnesium tanshinato B (MTB) purified from SMB stimulated the release of NO in human endothelial cells *in vitro*. Following treatment with magnesium tanshinato B, the cellular NOS activities were enhanced with a concomitant increase in the levels of cNOS protein mass.

The extract of *Uncaria rhynchophylla* (UR) was found by Kuramochi *et al* to relax the NE-precontracted rat aorta mainly through endothelium-dependent mechanism, and partially via endothelium-independent way. This relaxation produced by UR was long-lasting.
cium stores more selectively than calcium influx from extracellular space via voltage-dependent calcium channels using the rat’s descending thoracic aorta in vitro.

Hirsutine, an indole alkaloid found in UR was shown in previous study that it could not only inhibit calcium release but also increase calcium uptake into the calcium store as well as block the voltage-dependent calcium channel leading to a reduction of intracellular Ca\(^{2+}\) level in isolated rat[20].

Tetrandrine (TET) has a long history of use for the treatment of angina and hypertension in China[7]. It has been shown to block Ca\(^{2+}\) channels in various tissues as a non-selective calcium channel antagonist[25]. In addition, dehydroevodiamine (DeHE), a quinazolinocarboline alkaloid isolated from the fruit of Evodia rutaecarpa also need to be noted as its vasorelaxant effect involved everal mechanisms which include endothelium dependence, \(\alpha\)-adrenoceptor blockade, K\(^{+}\) channel activation apart from Ca\(^{2+}\) channel blockade in vitro[26].

**ANTI-ARRHYTHMIC EFFECT**

Many natural products show their effects on inhibition of ventricular tachycardia (VT), ventricular fibrillation (VF) and/or extrasystole during the period of I/R injury or AMI in animals. Pretreatment with magolol and honokiol significantly reduced the incidence and duration of ventricular arrhythmia including VT and VF in rats[2,3]. Such effects may in part be relevant to an increased NO synthesis. In a similar model, trilinolein reduced or completely suppressed not only the incidence and duration of VT but the number of ectopic beats as well[11].

Dauricine, an active compound found in Menispermum dauricum, ameliorate acute myocardial ischemia-induced VT and VF in anesthetized dogs[27]. The mechanism of its antagonistic effect lies in its inhibition of K\(^{+}\), Na\(^{+}\) and Ca\(^{2+}\) in ventricular myocytes [28]. Berberine could also significantly suppress the ventricular premature beats and VT in both animal experiments and clinical observation[29,30]. Neferine, isolated from the seeds of Nelumbo nucifera Gaertn, prevented the onset of re-entrant VT and sudden cardiac death after myocardial ischemic damage in vivo[31].

Oxymatrine, isolated from the genus Sophora, increased diastolic excitability threshold and lengthened effective refractory periods (ERP) in dogs after myocardial infarction[32]. But it had no effects on dispersion of ERP, VT and VF induced by programmed electric stimulation. In comparison, Crataegus oxyacantha presented contradictory results with regard to its anti-arrhythmic effects[33,34]. In a recent study, it aggravated rather than prevented arrhythmias after long-term application while protective effects were previously reported in rats after short-term as well as long-term oral treatment.

The same effects can also be seen in in vitro study. The inhibition of VT and VF could be seen in treatment with TET or RST extract but not FAN during regional I/R injury without further reducing ischemia-reduced heart rate and coronary artery flow[61]. Berbamine is very similar in structure to TET and seems to act in a virtually identical way on the cardiovascular system[7]. Pretreatment with sinomenine[35], a compound found in Sinomenium acutum, or TMPZ[36] could also reduce the incidence of VT and VF in isolated perfused rat heart subject to I/R injury. The latter also enhanced the release of PGI\(_2\) and diminished the outflow of TXB\(_2\). Other natural products having anti-arrhythmic protection effects are: hirsutine, EGB761, and dehydroevodia-mine (DeHE). Hirsutine showed anti-arrhythmic action on membrane potentials of rabbit sino-atrial node and guinea-pig right ventricle and left atrium in vitro[57].

It had direct effects on the action potential of cardiac muscle through inhibition of multiple ion channels, which might explain why its hypotensive effect was accompanied by negative chronotropic activity without reflex tachycardia. EGB761, a Ginkgo biloba extract, in combination with SOD could reduce the incidence of reperfusion-induced VF and VT in isolated rat hearts[38]. DeHE was shown to depress arrhythmias in calcium-overloaded guinea-pig cardiac myocytes through its inhibitory actions on the Na\(^{+}\)-dependent inward current, the transient inward current and, to a smaller extent, the L-type Ca\(^{2+}\) current. In the mean time, DeHE also had class III antiarrhythmic effect through a reduction of outward K\(^{+}\) currents across the sarclemma[39]. But the cause of the DeHE-induced bradycardia seemed unlikely to be due to antagonism of Ca\(^{2+}\) influx in isolated mouse hearts[40].

**IMPROVEMENT OF OTHER CARDIAC PARAMETERS**

Recent studies have suggested that the mitogen-activated protein kinases (MAPKs) may be implicated in apoptosis. p38 MAPK and c-Jun NH\(_2\)-terminal protein kinase (JNK/SAPK) are also called stress-regulated mitogen-activated protein kinases mediating apoptosis
after I/R injury whereas extracellular signal-regulated kinase (ERK) plays a role in protecting cardiomyocytes\textsuperscript{[41]}. Magnesium tanshione A, a component of SMB could abolish the elevation in SAPK activity in isolated rat hearts and prevented the induction of apoptosis in reperfusion injury\textsuperscript{[42]}. Similar effect can be seen in another natural product, Flos carthami (FC). Siow et al\textsuperscript{[43]} used an aqueous Flos carthami extract to investigate its effect on SAP kinase in IR rats both \textit{in vivo} and \textit{in vitro} and found that FC extract, which protected the isolated heart against arrhythmia caused by I/R injury, significantly inhibited SAPK activity via two mechanisms. One is that it affects the interaction of SAP kinase with c-jun. The other is that it inhibits the phosphotransferase reaction. The administration of the FC extract may lead to a modulation of the apoptotic effect induced by SAP kinase.

Ischemic myocardial injury initiates an acute inflammatory response in which polymorphonuclear leukocytes are major participants. Magnolol could significantly suppress \textit{N}-formylmethionyl-leucyl-phenylalanine-activated human neutrophil migration as well as expression of VCAM-1 resulting in reduced adhesion of leukocytes \textit{in vitro}\textsuperscript{[44,45]}. In cultured human umbilical vein endothelial cells, baicalein inhibited endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 expression induced by thrombin and phorbol myristate acetate\textsuperscript{[46]}. In terms of myocardial metabolism, pretreatment with Polygonum multiflorum Thunb [PME] extract or Crataegus extract in isolated rat heart models could significantly attenuate LDH release, suggesting a preservation of the cell membrane from I/R damage\textsuperscript{[47,48,49]}. Meanwhile, the latter lowered the level of lactate during ischemia as well as accelerated the reperfusion-induced recovery of the energy metabolism including decreasing the level of AMP and increasing the level of energy charge potential\textsuperscript{[49]}. EGB 761 also suppressed the leakage of LDH as well as diminished the decrease in myocardial ascorbate content concomitant with suppressing the increase of dehydroascorbate in cardiac I/R injury\textsuperscript{[50]}. Tetrahydropalmatine (THP) and its analogue were proved to ameliorate the increase of creatine kinase and aspartate aminotransferase in the serum of rats subject to I/R\textsuperscript{[51]}. Treatment with berberine in both anoxic and aerobic perfusion media in isolated rat hearts resulted in a significant reduction of creatine phosphokinase (CPK) release accompanied by amelioration of ultrastructural damage\textsuperscript{[51]}. Zhang and co-workers\textsuperscript{[52]} found similar results when they pretreated I/R rats with paenol. Puerarin, an isoflavone isolated from plants of the genus \textit{Pueraria}, has already been widely used in the treatment of angina and other ischemic diseases. In a clinical trial, it was shown that the frequency of angina events and myocardial oxygen consumption were reduced with improving abnormal rest ECG and increasing exercise duration\textsuperscript{[53]}. Such beneficial effects on myocardial function were consistent with previous studies in which puerarin preserved mitochondrial structure and decreased myocardial lactate production, oxygen consumption and CPK release after myocardial I/R injury in dogs\textsuperscript{[54,55]}. In addition, some reported that puerarin might play a role in regulating the imbalance of endothelin, rennin, and angiotensin II activity in patients with acute myocardial infarction\textsuperscript{[56]}. It is also necessary to be mindful of the protective effects of natural products on cardiac hemodynamics. Pretreatment with magnolol markedly enhanced the recovery of systolic wall thickening fraction 60 min after coronary artery reperfusion in anesthetized, open-chest rabbits\textsuperscript{[57]}. Berberine improved the impaired left ventricular function by its positive inotropic effect and mild systemic vasodilation\textsuperscript{[58]} and ameliorated the decreased cardiac output in dogs subject to IR injury\textsuperscript{[29]}. Scoparone could increase coronary flow and inhibit the depression of ST waves as an anti-anginal drug\textsuperscript{[59]}. Angelica sinensis (AS) increased the coronary blood flow with decreasing myocardial oxygen consumption\textsuperscript{[60]}. When AS was injected before I/R injury, the LVP and \textit{+/-dp/dt} of the rabbit heart were significantly higher than those in the control group\textsuperscript{[61]}.\

**ANTIOXIDANT EFFECTS OF NATURAL PRODUCTS**

Healthy cells can scavenge free radicals effectively by means of the antioxidant. In general, this is a dynamic relationship between reactive oxygen species (ROS) and antioxidants in the human body. However, when cells suffer I/R injury, the sudden generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. Natural antioxidants including superoxide dismutase (SOD), catalase and glutathione peroxidase are depleted accompanied by accumulation of ROS. In such situation, natural products can play an important role in two aspects: enhance the activity of original natural an-
Oxidants and neutralize ROS work by nonenzymatic mechanisms.

With regard to the first aspect, SMB shows potent effects\(^{41}\). It could reduce oxidant stress from MI by increasing the activities of hepatic-antioxidant enzymes, including superoxide dismutase, catalase, glutathione S-transferase, and tathione peroxidase (Tab 1). Other products including trilinolein, paenol from Paeonia Radix, sinomenin from Sinomenium acutum, and Menispernum dauricum could also increase SOD in rats\(^{63}\) or incubation at similar concentration in rat aortic smooth muscle cells\(^{66}\), the activity and mRNA levels of SOD were increased. However, when prolonging the time of incubation to seven days, these parameters were lowered in a dose-dependent manner. These phenomena imply that low dosage of trilinolein given short term stimulates endogenous antioxidant (SOD). By contrast, after long-term administration, it could replace the action of SOD in terms of an OFR scavenger. In addition, Polygonum multiflorum Thunb was found to have myocardial protection associated with an enhancement in myocardial glutathione antioxidant status impaired by IR-induced oxidative stress\(^{47}\).

In an on-going project, initial screening of whole extracts of Rhizoma ligustici (RL), Herba leonuri (HL)

Tab 1. Anti-oxidant status of the liver in DanShen-, saline- and ramipril-treated rats. n=6. Mean±SD. *P<0.01 vs corresponding saline group. ‡P<0.01 vs ramipril-treated group.

<table>
<thead>
<tr>
<th>Enzyme A (kU/g protein)</th>
<th>DanShen</th>
<th>Saline</th>
<th>Ramipril</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>0.674±0.016(±)</td>
<td>0.58±0.03</td>
<td>0.630±0.019</td>
</tr>
<tr>
<td>SOD</td>
<td>16.2±0.3(±)</td>
<td>10.6±0.3</td>
<td>8.9±0.7</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>0.453±0.013(±)</td>
<td>0.353±0.012</td>
<td>0.248±0.012</td>
</tr>
<tr>
<td>GST</td>
<td>5.23±0.11(±)</td>
<td>4.0±0.22</td>
<td>2.6±0.10</td>
</tr>
</tbody>
</table>

All assays were performed in duplicate at 25 °C. One unit of SOD is defined as the amount of enzyme necessary to inhibit the superoxide-dependent oxidation of pyrogallol 10 mmol/L; 1 unit of catalase (CAT) defined as mmol H\(_2\)O\(_2\) decomposed/min; 1 unit of glutathione peroxidase (GSH-Px) is defined as 1 μmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) converted to NADP\(^+\)/min; 1 unit of glutathione S-transferase (GST) is defined as 1 μmol CDNB converted to CDNB-glutathione/min.

and Radix Achyranthis bidentatae (RAB) has proved very interesting. RL, HL and RAB have been analysed for scavenging of peroxynitrite (ONOO\(^-\)), hydrochlorite (HOCl), hydroxyl radical (‘OH), 2,2’-azino-bis(3-ethylenbenz-thiazoline-6-sulfonic acid) (ABTS\(^•+\)) and diphenyl-1-pclyrylhydrazyl (DPPH) as well as inhibition of Fe-induced lipid peroxidation and in each assay antioxidant activity greater than that, on a molar basis, than either ascorbic acid or Trolox, a water soluble analogue of vitamin E. We have shown that these compounds contain moieties other than phenolics that are responsible for the antioxidant activity (Fig 1).

Free radicals alter the structural and functional integrity of cells by a variety of mechanisms, including lipid peroxidation, sulfhydryl oxidation, proteolysis, and shearing of the nuclear material. Most research on natural products in recent years concentrates on the inhibition of lipid peroxidation.

Among these natural products, magnolol and honokiol show antioxidant effects 1000 times higher than that of alpha-tocopherol when oxygen consumption and MDA production were measured to quantitate lipid peroxidation in isolated rat heart\(^{69}\). But magnolol and honokiol’s free radical scavenging activities were less potent than alpha-tocopherol, consistent with another experiment showing that when compared with saturated and unsaturated lipid compounds (trilinolein, catechin, trolox, et al) by means of enhanced chemiluminescence\(^{1}\). According to this data, trilinolein has the most post potent antioxidant effect, which in part explains its protection of mitochondrial ultrastratute, and improvement of erythrocyte deformability in vivo. The extract of Curcuma longa (CL)\(^{66,67}\) was tested for its effects on atherosclerosis prevention, and found that it could decrease the susceptibility of low density lipo-protein (LDL) to lipid oxidation in atherosclerotic rabbits and lower the abnormally high values of human-plasma fibrinogen and apo B/ apo A ratio in clinical trials. Similar effect of preventing LDL from oxidation could also be seen in the treatment with SMB and its main components MTB and tanshinone II-A in vitro\(^{66-70}\).

Since superoxide, hydrogen peroxide, and hydroxyl ions are the three main free radicals involved in initiating a chain reaction of lipid peroxidation, some natural products have the ability to directly scavenge this kind of free radicals. Danshensu, an important compound of SMB, working as a preventive antioxidant, could scavenge superoxide anions generated from xanthine-xanthine oxidase system and protect the myocardial...
mitochondrial membranes from lipid peroxidation. And tanshinone, a chain-breaking antioxidant, scavenged the lipid free radicals generated from lipid peroxidation of the myocardial mitochondrial membranes. Shao et al. found that baicalein could directly scavenge superoxide, hydrogen peroxide, and hydroxyl radicals, and significantly attenuate cell death after I/R in cardiomyocytes. Its chemical structure, o-di-hydroxyl group in the A ring, plays a key role. But recent study showed that baicalein and baicalin posed a different pathological pathway. The antioxidant function of baicalin was mainly based on scavenging superoxide radical whilst baicalein was a good xanthine oxidase inhibitor. Schisanhenol isolated from Fructus schizandrae scavenged hydroxyl radicals induced by adriamycin in rat heart mitochondria. And a recent study showed that some paeonol glycosides exhibited more potent radical scavenging effects than α-tocopherol. Protykin, an all-natural extract of trans-resveratrol (20%) and emodin (10%) derived from the dried rhizome of Polygonum cuspidatum, also possessed potent peroxy and hydroxyl radical scavenging activities in isolated rat hearts.

It should be noticed that two natural products present their antioxidant effects indirectly. One is Ginkgo biloba (Gb) and ginkgolides, which are terpenoids found in its leaves. Using hemodynamic and electron spin resonance (ESR) analyses, both in vivo and in vitro, Liebgott and co-workers found that part of the cardioprotection afforded by Gb extract involved a mechanism independent of direct scavenging of free radicals. This conclusion is consistent with a previous study showing that they appeared to inhibit free radical formation rather than directly scavenge free radical. The other is puerarin and Radix puerariae. Both of them were studied in live rats. Unlike Radix puerariae extract, puerarin seemed to act as an enzymatic inhibitor of the oxygen radical production mediated by the peroxidase/H2O2/luminol/enhancer radical reactions, rather than as a true antioxidant.

According to the present knowledge, many natural products are involved in direct or indirect inhibition of lipid peroxidation. Aside from the natural products I mentioned above, tetrahydroprotoberberines decreased...
malondialdehyde (MDA) content, and xanthine oxidase activity and scavenge hydroxyl free radicals in vitro. Ginsenosides and saponins from Panax ginseng decreased lipid peroxidation concomitant with increasing 6-keto-PGF,α in vivo. Camellia sinensis, with flavan-3-ol tannins inhibited lipid peroxidation in rat heart mitochondria. Crataegus extracts showed cardioprotective effects via their radical scavenging and elastase inhibitory activities. Paeoniae Radix and Moutan cortex, both from the Paeoniae paoniae family, could suppress phenylhydroquinone-induced oxidative DNA cleavage.

CONCLUSION

In conclusion, the effects of most natural products on ischemic heart diseases are multiple and complex. In animal models, they show their abilities to improve myocardial function, promote capillary growth, relax vascular smooth muscle or myocardial contractility, inhibit arrhythmia induced by I/R injury and reduce myocardial infarct size. Meanwhile, compared to calcium antagonists or angiotensins converting enzyme inhibitors, some of them also have a unique profile, antioxidant activity, such as trilinolein, magnolol, and danshensu et al, which further strengthen their status in the treatment of ischemic heart diseases. All these effects partially explain why traditional Chinese medicines could have been widely used in Eastern countries for thousand years.

However, the more we study their mechanisms, the more complex they appear. For example, the relationship among apoptosis, ROS, and NO is very subtle in pathophysiology. It plays a key role in ischemic heart diseases, especially in myocardial infarction. Several natural products have already been demonstrated to be involved in this relationship. Flos carthami and MTB inhibited apoptosis by means of reducing SAP kinase activity; Gb extract, magnolol, and honokiol suppressed excessive NO production injury caused by iNOS expression. And many products mediate the balance between free radicals and NO via antioxidant activity. Further studies, especially clinical trials, still need to be done. Meanwhile, before we obtain more conclusive data on clinical efficacy and safety, herbal medications still need to be used prudently when accompanied with other Western drugs.

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