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Cardiovascular actions of Radix Stephaniae Tetrandrae: a comparison with its main component, tetrandrine

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KEY WORDS Radix Stephaniae Tetrandrae; tetrandrine; fangchinoline; calcium; arrhythmia; myocardial infarction; deoxycorticosterone; hypertension

ABSTRACT

A comparison of the cardiovascular actions of the extract of Radix Stephaniae Tetrandrae (RST), the root of a Chinese herb *Stephania tetrandra* S Moore, in rats with those of tetrandrine (Tet), the best known active component of RST was reviewed. The RST extract inhibits Ca^{2+} influx into the myocyte and reduces protein release during reperfusion with a Ca^{2+} containing solution following perfusion with a Ca^{2+} free solution (Ca^{2+} paradox), and arrhythmia during reperfusion in the isolated perfused heart. It also reduces the infarct size induced by ischemia/reperfusion *in vitro* and *in vivo*. In addition, the RST extract suppresses elevation of arterial blood pressure in DOCA-salt hypertensive rats. It does not further reduce the heart rate and coronary flow significantly during myocardial ischemia. The effects are similar to those of Tet. When compared with the same doses of Tet alone, the RST extract, of which 9 % is Tet, produces equally potent effects on infarction, arrhythmias, coronary flow and heart rate, and has a greater inhibitory effect on protein release during Ca^{2+} paradox. The combination at 1:1 ratio of Tet and fangchinoline (Fan), another main component, which constitutes 6 % of the RST extract and has no significant effects on the heart, produces comparable effects on protein release during Ca^{2+} paradox as Tet alone. The observations suggest that the efficacy of the RST extract cannot be accounted for by Tet alone. Some of the effects may be due to an interaction between the components of the extract. The RST extract also produces similar effects as verapamil, a prototype Ca^{2+} channel antagonist widely used in the treatment of ischemic heart diseases and hypertension, except that verapamil, at 1 μmol/L, a concentration that produces similar cardiac effects as the RST extract, further reduces heart rate significantly during ischemia. So the RST extract may be a therapeutically better agent in the treatment of ischemic heart diseases and hypertension than Ca^{2+} channel antagonists because of the absence of the inhibitory effect on heart rate during myocardial ischemia.

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INTRODUCTION

Radix Stephaniae Tetrandrae (RST), the root of a Chinese herb *Stephania tetrandra* S Moore, was described as a diuretic, expectorant and cathartic folk medicine by the famous Chinese herbalist Li Shi-Chen in his book "Compendium of Materia Medica" four hundred years ago. It was used to treat water retention in the ancient time[1]. The natural root of RST contains various biological bis-benzylisoquinonline alkaloids such as tetrandrine (Tet), fanchinoline (Fan), cyclanoline, oxofangchirine, stephanthrine, cyclanine, 2-*N*-methyltetrandrine etc. The alkaloids constitute approximately 2.3 % of the total natural RST root content[2]. Of all the alkaloids, Tet and Fan are two major components of RST as they constitute 1 % and 0.5 % of the total natural RST root content, respectively[3-5].

CARDIOVASCULAR EFFECTS OF TET

Tet, the most abundant component in RST, has drawn most attention. The first pharmacological and toxicological study of Tet was published in 1937[6], which has since been quoted worldwide. Tet was first shown to act as a calcium (Ca^{2+}) channel antagonist on

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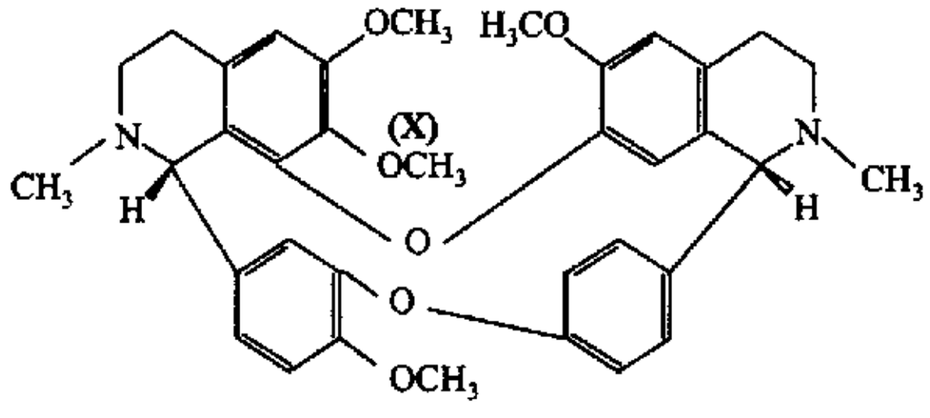
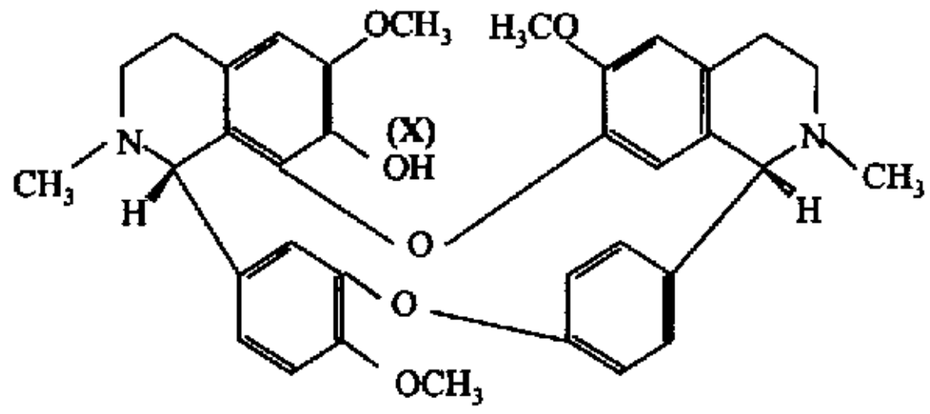


Tetrandrine



Fangchinoline

myocardium[7,8] and on vascular smooth muscle[9,10]. Subsequently, Tet was demonstrated to inhibit Ca^{2+} influx via both L- and T-type voltage-operated Ca^{2+} channels in ventricular cells[7] and in vascular smooth muscle[11], as well as via non-voltage-operated Ca^{2+} entry in vascular smooth muscle[12]. A binding study also showed that Tet elevates the binding of [^{3}H]-nitrendipine to the Ca^{2+} entry blockers receptor complex of L-type Ca^{2+} channel in cardiac sarcolemmal membrane vesicles[13], supporting that Tet may bind to the L-type Ca^{2+} channel in the heart. Further studies showed that Tet also inhibits Ca^{2+} release from its intracellular stores in addition to inhibition of Ca^{2+} entry from the extracellular medium[12,14].

In addition to inhibition of Ca^{2+} influx and release Tet has modulatory effects on other targets, such as Ca^{2+}-activated K^+ channels, Na^+-K^+ ATPase, α-adrenoceptors and protein kinase C, and membrane lipids[15]. It should be noted that Tet is a non-selective Ca^{2+} channel antagonist, and its action on Ca^{2+} entry may be different in different cell types[14].

MULTIPLICITY OF TET ACTIONS

Further studies demonstrated that Tet reduces heart rate and contractility, and shortens action potential duration[8]. It also causes vadodilation[8,10]. More importantly, it reduces infarct size induced by myocardial ischemia[16,17], and protein release following perfusion of a Ca^{2+} free solution, a phenomenon called "Ca^{2+} paradox"[18]. In addition, it reduces arrhythmia[19] and arterial blood pressure[20]. So it has cardioprotective, antiarrhythmic and antihypertensive effects. In China it is being used for the treatment of angina and hypertension[21,22]. It is believed that the inhibitory effect on Ca^{2+} influx is the basis of the cardiovascular action and therapeutic efficacy of Tet.

The inhibitory effect on Ca^{2+} influx may also account for other actions of Tet such as the anti-inflammatory[23] and immunosuppresive[24] effects. It may also be linked to inhibition of neutrophil adhesion and activation, which abolishes subsequent infiltration and production of reactive oxygen species, one of the causes of ischemia/reperfusion injury[25]. A recent report also showed that inhibition of NO production by the endothelial cells may result from blockade of Ca^{2+} release-activated Ca^{2+} channels[26].

It is believed that with Tet as its main component RST may produce the same cardiovascular effects that Tet produces. We have recently studied the cardiovascular actions of the extract of RST and compared its effects with those of Tet and verapamil, a prototype calcium channel antagonist. We have also compared the effects of Tet with those of combination of Tet and Fan, another main component of the extract, with 50 % of each. This review summarizes the main findings obtained primarily from our laboratory. The RST extract used was generously given by Prof CF CHEN, Director of the National Institute of Chinese Medicine, Taiwan. 9 % and 6 % of the extract was constituted of Tet and Fan, respectively, according to Dr J SHAN of CV Technology, Inc, Canada.

CARDIAC ACTIONS OF RST EXTRACT

Electrically-induced intracellular calcium ([Ca^{2+}]$_i$) transient The electrically-induced [Ca^{2+}]$_i$ transient actually represents influx of Ca^{2+} upon membrane depolarization resulting from electrical stimulation, which triggers a sudden release of Ca^{2+} from the sarcoplasmic reticulum (SR) via a Ca^{2+}-induced-Ca^{2+}-release mechanism[27]. Previous studies have shown that in similar experimental conditions as in the study the electrically-induced [Ca^{2+}]$_i$ transient *is directly proportional* to contraction[28,29], indicating that the transient may also

represent the contractility. In single isolated ventricular myocytes, the RST extract at 2 – 200 mg/L concentration-dependently inhibits the electrically-induced $[Ca^{2+}]_i$ transient (Tab 1), an effect similar to that of Tet and verapamil[30]. The IC_{50} value was 10.3 mg/L. We have shown that the RST extract inhibits the Ca^{2+} influx via the L-type channel without affecting the caffeine-induced $[Ca^{2+}]_i$ transient, which is an indication of Ca^{2+} content in SR[31,32]. The observation indicates that the RST extract at this concentration range does not affect mobilization of Ca^{2+} from the intracellular Ca^{2+} stores. Therefore the inhibitory effect of the RST extract on electrically-induced $[Ca^{2+}]_i$ transient is mainly due to the inhibition of influx of Ca^{2+}. This is in agreement with our observation with a patch clamp study that the RST extract inhibits Ca^{2+} influx via the L-type Ca^{2+} channel (unpublished result). For the studies on other parameters, 18.5 mg/L was chosen as this was the minimal concentration to produce optimal effects in the study on electrically-induced $[Ca^{2+}]_i$ transient.

Protein release during Ca^{2+} paradox There was a significant increase in the release of protein during reperfusion with a Ca^{2+} containing solution following perfusion with a Ca^{2+} free solution in the isolated perfused rat heart, an indication of ischemic cardiac injury[33,34]. Our research demonstrated that RST extract reduced the protein release to less than 1/3rd of the value of vehicle control (Tab 1).

Heart rate and coronary artery flow during myocardial ischemia When the isolated perfused rat heart was subjected to regional ischemia by ligation of the coronary artery, the heart rate and coronary flow were reduced by approximately 20 % and 40 %, respectively. The RST extract at 18.6 mg/L seemed to cause further reduction both in heart rate and coronary flow, but these were not statistically significant (Tab 2).

Tab 1. Cardiac effects of Tet or Fan alone, combination of Tet and Fan with half each, and RST extract.

	Vehicle	Tet	Fan	Tet + Fan	RST extract
Electrically-induced $[Ca^{2+}]_i$ transience (% Vehicle)	100	39	88 (NS)	49	55
Total protein release during Ca^{2+} paradox (% Vehicle)	100	56	94 (NS)	59	47
Infarct size/risk area (% Vehicle)	100	60	91 (NS)	72	54
Arrhythmias (% Vehicle)	100	51	91 (NS)	63	49

All figures are percentage of the vehicle control, which is 100 %. All are significantly different from the control except those with NS. In all studies the concentration was 30 mmol/L (18.6 mg/L) for Tet and Fan alone, and 15 mmol/L each for combination of the two drugs. The concentration of RST extract was 18.6 mg/L. All experiments were performed 6 – 11 times. All data were obtained from isolated perfused adult rat heart except for those on electrically induced $[Ca^{2+}]_i$ transient, which was measured in single isolated ventricular myocytes of adult rats. Ventricular myocytes, isolated from the adult rat heart, were loaded with Fura 2-AM, a calcium indicator. The myocytes were then subjected to electrical field stimulation and the $[Ca^{2+}]_i$ transient generated was detected by spectrofluorometry. Isolated rat hearts were perfused with Krebs ringer solution. For Ca^{2+} paradox experiments, the heart was perfused with a Ca^{2+} free solution for 8 min, which induces an increase protein release during subsequent reperfusion with a Ca^{2+} containing solution for 20 min. For determination of infarct size and arrhythmia induced by ischemia, the isolated perfused rat heart was subjected to regional ischemia for 30 min by ligation of the coronary artery followed by reperfusion for 120 min. Arrhythmia was monitored for the first 10 min into reperfusion while infarct size determined at the end of reperfusion.

Tab 2. Effects of Tet or Fan alone, combination of Tet and Fan with half each, RST extract and verapamil on heart rate and coronary flow during myocardial ischemia.

	Pre-ligation	Vehicle	RST extract	Verapamil (μmol/L) 0.1	1	10
Heart rate (% pre-ligation)	100	83 (NS)	92 (NS)	63 (NS)	22	12
Coronary flow (% pre-ligation)	100	66 (NS)	59 (NS)	59 (NS)	57 (NS)	39

All figures are percentage of the pre-ligation control, which is 100 %. All are significantly different from the control except those with NS. All experiments were performed 6 – 11 times. Heart rate and coronary flow during ischemia were determined in the isolated perfused rat heart subjected to regional ischemia for 30 min by ligation of the coronary artery. The concentration of RST extract was 18.6 mg/L. Verapamil 1 mmol/L produced the similar effects on infarct and arrhythmia as RST extract at 18.6 mg/L did.

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Infarct caused by myocardial ischemia *in vivo* and *in vitro* Myocardial ischemia by coronary artery ligation induced myocardial infarction in the isolated perfused rat heart and in anaesthetized rat. The RST extract at 18.6 mg/L significantly reduced the infarct size in the isolated perfused rat heart by approximately 55 % (Tab 1). Similarly, in anaesthetized rats, the RST extract also reduced the infarct size caused by coronary artery ligation (data not shown).

Cardiac arrhythmias during reperfusion following myocardial ischemia Severe ventricular arrhythmias occurred mainly during early reperfusion following myocardial ischemia. The RST extract significantly reduced the severity of ischemia/reperfusion induced arrhythmias. As shown in Tab 1, the RST extract at 18.6 mg/L reduced the incidence of reperfusion induced arrhythmias by almost 50 %.

Antihypertensive effect Our preliminary study showed that the systolic blood pressure measured by the Tail-Cuff method, was elevated significantly in the rat by removal of left kidney followed by daily administration of deoxycorticosterone acetate, and drinking of 1 % sodium chloride solution (DOCA-salt hypertensive rat)[35,36]. Feeding of the RST extract at 150 mg/kg per day significantly attenuated the elevation in arterial blood pressure and reduced the mortality rate. The anti-hypertensive effect occurred as early as 2 days and lasted at least for 9 weeks after starting the administration of the extract.

In summary the RST extract produces effects qualitatively similar to Tet. We next observe whether Tet is solely responsible for the effects of the extract.

COMPARISON BETWEEN RST EXTRACT AND TET

In the practice of traditional Chinese medicine, a formula comprising a mixture of compounds is prescribed. It is believed that the therapeutic efficacy of the mixture is not only due to the action of individual components alone, but more importantly the interaction and/or additive actions of different components. It is therefore hypothesized that the mixture may produce greater effects than the individual compounds. To test the hypothesis, the effects of equivalent concentrations of the RST extract and Tet were compared. The RST extract at 18.6 mg/L produced comparative effects on infarct size, arrhythmia, coronary flow and heart rate as Tet at the same concentration (Tab 1,2). The RST extract had a greater inhibitory effect on protein release during Ca^{2+} paradox than Tet (Tab 1). So the effects of RST extract cannot be accounted for by Tet alone, which constitutes only 9 % of the extract. Other compounds in RST extract also contribute to its effects. This is in agreement with a previous observation that the crude extract of *Phyllanthus emblica* fruits affords more pronounced protection against the adverse cellular effect of environmental toxicants than ascorbic acid, its major effective single compound[37,38]. Another study also illustrates that the protective action against the clastogenic effects of the crude extract from certain *Indian spinach* leaf was greater than that of its main effective constituent chlorophyll alone[39]. It is hence believed that the higher efficacy of the crude extract can be attributed to the synergistic interaction of the main component with other natural ingredients present in the crude extract[37,38] or due to the combined action of all ingredients of the crude extract, rather than the main component alone[37-39].

COMPARISON BETWEEN COMBINATION OF TET AND FAN AND TET ALONE

In order to determine whether there is an interaction between different compounds, the effects of Tet were compared with those of combination of Tet and Fan, a structurally similar alkaloid, which constitutes 6 % of the RST extract, with 50 % of each in the mixture. Fan, which only differs from Tet with an $-OH$ group in the isoquinoline portion (X position) instead of $-OCH_3$ (Fig 1), had almost insignificant effects in any of the parameters, which Tet affected significantly. The lack of cardiovascular effects of Fan are believed to be mainly due to a low potency of Fan in blocking Ca^{2+} influx and release[4,5,40], which was suggested to be due to the $-OCH_3$ group at the X position[12,41]. It is reasoned that if the combination produced a greater effect than that expected from the composition having 50 % of each component, the result would be taken as to suggest that the two individual compounds may be interacting synergistically with each other, leading to an enhanced effect. The effects of combination of Tet and Fan were comparable to those of Tet on protein release during Ca^{2+} paradox (Tab 1), although Fan itself had almost no inhibitory effect on the protein release during Ca^{2+} paradox. The observation suggests that there may be an interaction between the two compounds. On the other hand, the effect

of combination of Tet and Fan was much smaller than that of Tet alone on infarct, indicating absence of any interaction between these two compounds. The observation suggests specific interaction between individual components for certain actions.

Interaction between two or more compounds is not uncommon. For example, 7-*O*-ethylfangchinoline (7-*O*-Fan), a derivative of Tet itself reduces the arterial blood pressure of the spontaneously hypertensive rat only slightly, but together with trichloromethinizide, a thiazine diuretic, which itself has no significant effect on arterial blood pressure either, produces a very significant antihypertensive effect[42]. Similarly, the antagonistic effect of combination of four components fractionated by HPLC from black tea on viral infections is markedly greater than the sum of the activities of these four components individually[43].

So the greater potency of an extract may result at least in part from interaction of individual components. In the case of RST extract, regarding Ca^{2+} paradox, the greater potency of the extract may be at least partly due to the interaction or synergism between Tet and Fan.

COMPARISION BETWEEN RST EXTRACT AND VERAPAMIL

To determine the therapeutic value of the RST extract, the effects of the extract were compared with those of verapamil, a prototype Ca^{2+} channel antagonist[44]. Like the RST extract, verapamil reduced the electrically-induced $[Ca^{2+}]_i$ transient in the ventricular myocytes, and the infarct size and the severity of arrhythmias (Tab 1) induced by myocardial ischemia and reperfusion in the isolated perfused rat heart. Unlike the RST extract, at the concentration range 0.1 μmol/L, which produced quantitatively comparable effects on infarct and arrhythmia as 18.6 mg/L RST extract, it further reduced the heart rate markedly (Tab 2), which is undesirable.

CONCLUSIONS

RST extract produces cardiovascular actions by virtue of its inhibitory effect on Ca^{2+} with an efficacy similar to Tet alone. There may be an interaction between Tet and other components such as Fan. That the RST extract has cardioprotective, anti-arrhythmic and anti-hypertensive actions without the inhibitory effect on heart rate may make it a useful therapeutic agent for the treatment of ischemic heart diseases and hypertension.

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