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Antihypertensive and anti-arrhythmic effects of an extract of *Radix Stephaniae Tetrandrae* in the rat

X.-C. Yu, S. Wu, C.-F. Chen, K. T. Pang and T. M. Wong

Abstract

In this study, we determined the effects of an extract *of Radix Stephaniae Tetrandrae* (RST) on arterial blood pressure and heart weight in deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats. We also determined the effects of the extract on arrhythmia and infarct induced by myocardial ischaemia and reperfusion in anaesthetized rats. We further compared the effects of the extract with those of tetrandrine, which makes up 7% of the extract and is known to act as a calcium-channel antagonist, and verapamil, a prototype calcium-channel antagonist. Treatment with RST extract returned the arterial blood pressure, cardiac compliance and coronary flow towards normal, and reduced right ventricular hypertrophy in the DOCA-salt hypertensive rat. In the anaesthetized rat, the RST extract reduced arrhythmia and infarct size induced by myocardial ischaemia and reperfusion; the effects were similar to those of tetrandrine and verapamil. The findings indicate that the RST extract acts like a calcium-channel antagonist. It may be used in the treatment of cardiovascular diseases, as are the calcium-channel antagonist and tetrandrine. More interestingly, the effects of the RST extract were of the same potency as tetrandrine. Since only 7% of the extract was tetrandrine, the observation indicates that tetrandrine was not the only component that was responsible for the actions of the extract.

Department of Physiology, Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

X.-C. Yu, S. Wu, T. M. Wong

National Research Institute of Chinese Medicine, Taiwan

C.-F. Chen

CV Technologies Inc., Alberta, Canada

K. T. Pang

Correspondence: T. M. Wong, Department of Physiology, Faculty of Medicine, The University of Hong Kong, 4/F Laboratory Block, 21 Sassoon Road, Hong Kong SAR, China. E-mail: wongtakm@hkucc.hku.hk

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Introduction

Radix Stephaniae Tetrandrae (RST) is the root of a Chinese herb, *Stephania* S. Moore. It was described as a diuretic, expectorant and cathartic folk medicine by Li Shi-Chen, a famous Chinese herbalist, in his book – *Compendium of materia medica* – four hundred years ago. One of the active compounds extracted from RST is tetrandrine, a bis-benzylisoquinoline alkaloid. Tetrandrine binds to the receptor complex of the L-type Ca^{2+} channel (Felix et al 1992) and inhibits the influx of Ca^{2+} via the L-type (Kwan & Wang 1993; Kwan 1994; Liu et al 1995; Wang et al 1997) and T-type (Leung et al 1996) Ca^{2+} channels. These observations suggest that tetrandrine may act as a Ca^{2+}-channel antagonist. In our previous in-vitro studies, both the RST extract and tetrandrine had cardioprotective effects (Wu et al 2001; Yu et al 2001). Since tetrandrine has already been used in the treatment of cardiovascular diseases such as hypertension and arrhythmias, we believe that the RST extract might also be used in the treatment of these disorders. To evaluate its therapeutic efficacy, the antihypertensive and anti-arrhythmic effects of the RST extract in-vivo need to be determined.

In our previous studies (Wu et al 2001; Yu et al 2001), we also found that the extract of RST produced the same effects, with similar or even greater potency, as an equal weight of tetrandrine. The observation suggests that tetrandrine may not be the only component responsible for the effects of the RST extract. Interaction of different components may contribute to its effects. Whether this also applies in-vivo needs further study.

In this study, we first determined the effects of the RST extract on arterial blood pressure and heart weight in deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats. We also determined the effects of the extract on arrhythmia and myocardial infarct induced by ischaemia and reperfusion in rats. In addition to determining the effects of the extracts and tetrandrine, we compared the effects of the extract with those of tetrandrine and verapamil, a prototype Ca^{2+}-channel antagonist. The extract used

contained tetrandrine 7%, fanchinoline 3.2% and cyclanoline 4.9% as the main components, according to determination by HPLC. Results from the study showed that the RST extract produced antihypertensive, antihypertropic, anti-arrhythmic and cardioprotective effects in-vivo as did tetrandrine and verapamil, agents that block Ca^{2+} channels. The effects of the extract may not be due to tetrandrine alone.

Materials and Methods

Drugs and chemicals

The RST extract was provided by the National Research Institute of Chinese Medicine, Taiwan. Tetrandrine 7.0%, fanchinoline 3.2%, cyclanoline 4.9%, oblongine 0.4%, and very small percentage of some other components, such as menisine and menisidine, were contained in the RST extract as analysed with Hitachi HPLC system (Shen et al 1999). The RST extract was dissolved in dimethyl sulfoxide (DMSO) and further diluted with Krebs ringer solution before each experiment. Tetrandrine was purchased from the Chinese Institute for Drug and Bioproduct Control. It was first dissolved in 0.1 M HCl as stock and then further diluted with Krebs Ringer solution before each experiment. The final concentrations of HCl and DMSO in the experimental solutions were $< 0.1\%$, at which concentration no effects were observed in the test systems. For gastric feeding, drugs were put in 0.5 mL of water, which was fed to the stomach via a gastric tube. The concentrations of the RST extract (Yu et al 2001), tetrandrine (Xu & Rao 1995; Wu et al 2001) and verapamil (Yu et al 2001) used in the present study were based on previous studies. Deoxycorticosterone acetate (DOCA), Tween-80, sodium carboxymethylcellulose (carmellose-Na) and verapamil were purchased from Sigma. DOCA was dissolved in Tween-80, carmellose-Na in saline and verapamil in distilled water.

Animals

Adult male Sprague-Dawley rats were used. Their use was approved by The Committee on the use of Live Animals in Teaching and Research, The University of Hong Kong. All rats were housed under conditions of constant room temperature ($25 \pm 1\,^{\circ}C$) and humidity (60–65%), with a 12-h light–dark cycle and had free access to food and water.

DOCA-salt hypertensive rat model and drug treatment

Sprague-Dawley rats, 250–280 g, were randomly divided into five groups as follows: normal control rats were given normal feed and water throughout the experimental period; DOCA-control rats received DOCA-salt model treatment for the first 10 weeks, and were then given vehicle (saline) for the next 10 weeks; DOCA-Tet rats received DOCA-salt treatment for the first 10 weeks, and were then fed with tetrandrine at $50\,mg\,kg^{-1}$ per day for the next 10 weeks; DOCA-RST low and high dose rats received DOCA-salt treatment for the first 10 weeks, and were then fed with the RST extract at 50 or $150\,mg\,kg^{-1}$ per day for the next 10 weeks.

The DOCA-salt treatment included removal of the left kidney followed by subcutaneous injections of $10\,mg\,kg^{-1}$ DOCA-salt (suspended in 1% NaCl with carboxymethylcellulose sodium and polysorbate 80) twice a week for 9 weeks, starting from the 2nd week after the operation (Xu & Rao 1995; Bianciotti and De-Bold 2000; Muthalif et al 2000). Starting from the 10th week after removal of the left kidney, the rats were fed with tetrandrine or RST extract, or same volume of saline, via a gastric tube once per day for another 10 weeks. Throughout the whole experimental period of 20 weeks, rats were given 1% NaCl to drink.

Determination of systolic blood pressure

The systolic arterial blood pressure was measured in conscious rats once per week throughout the experiment with a tail-cuff method. Only the systolic pressure was measured and it was used as an index of the arterial blood pressure because the diastolic pressure was not stable enough. Before measurement signal, rats were maintained at $35\,^{\circ}C$ for 5–10 min in a specially constructed chamber. The blood pressure signal was measured and recorded using a transducing set-up (IITC, BD111/112; Life Sciences, USA) connected to a computer. In each measurement the average of three readings was used as one entity. All blood-pressure data were recorded and stored in the Software BPMON (IITC Blood Pressure Monitor data view program, Version 2.30; IITC Life Science, USA).

Measurement of body weight and wet weight of heart and right kidney

The body weight of the rats was determined every week throughout the experiment. At the end of the experimental period, rats were sacrificed and hearts removed for determination of pressure–volume (P-V) relationship in the Langendorff isolated perfused heart preparation. After determing the P-V curve, the wet weights for each heart, left and right ventricle, and ventricular septa were determined. The right kidney was removed and wet weight measured immediately after the rats were killed.

Pressure–volume (P-V) curve and compliance of left ventricle

After the heart had been mounted in the Langendorff apparatus, it was perfused with a Krebs Ringer solution at a constant perfusion pressure of 80 cm H_2O. The coronary flow was collected during 10 min of the stable perfusion and expressed as the mean volume per min ($mL\,min^{-1}$) as described previously (Yu et al 2001). The relationship between left ventricular end-diastolic pressure (LVEDP, mmHg) and left ventricular balloon volume (mL) was determined as described previously (Horowitz et al 1986; Solomon et al 1998). Briefly, after a stable perfusion, a small cannulated water-filled latex balloon was inserted into the left ventricle via the left atrium. The pressure in the balloon was determined through a polyethylene tube connected to a pressure transducer, and the signal was recorded on a polygraph

(Gould TA240S, UK). The collapsed balloon was slowly filled with saline to obtain an LV diastolic pressure of 0 mmHg, which corresponded to a volume of approximately 0.1 mL saline in our studies. Then the balloon volume was increased in steps of 0.05 mL to reach 0.4 mL. The pressure for each volume load was measured using a micro-volume pressure transducer. A pressure–volume (P-V) curve was then generated.

Regional ischaemia/reperfusion in anaesthetized rats

Sprague-Dawley rats, 250–350 g, were anaesthetized by intraperitoneal administration of pentobarbital sodium (60 mg kg^{-1}) and were artificially ventilated. The right femoral artery was cannulated with a polyethylene tube, which was connected to a pressure transducer (Nihon Cohden MPU-0.5A) for the measurement and recording of the systemic arterial pressure using a myograph system (DMT wire myographs & PowerLab data acquisition system; PowerLab, AD Instruments). The right femoral vein was cannulated for drug infusion. A left thoracotomy was performed 2 cm from the sternum to expose the heart at the fifth intercostal space. A 2-0 silk thread was passed around a prominent branch of the left coronary artery close to the place of origin with a taper needle. After the surgical procedure, the rat was allowed to stabilize for 15 min. Any rat exhibiting arrhythmias during this period was sacrificed and discarded. Regional ischaemia was produced by ligating the coronary artery for 30 min, after which the ligation was released, which produced reperfusion. The duration was 120 min. Since arrhythmia was frequent and severe during the early reperfusion, it was determined in the first 10 min after reperfusion. All the drugs were infused just before ligation of the left coronary artery.

Measurement of risk zone and infarct size

Determination of infarct size was as described previously (Yu et al 2001). Briefly, upon completion of experiment, the heart was removed immediately and perfused retrogradely with a Krebs Ringer solution at 36 °C. Then 0.25% Evans blue was infused into the coronary system. The heart was then frozen and cut into 2-mm slices. After removal of the right ventricle and the connective tissue, the slices were incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTZ) in a buffer at pH 7.4 for 15 min at 37 °C, and then immersed in 10% formalin overnight. Photographs of the slices were taken and digitized, and some enhancement of the image was achieved using Adobe Photoshop (Version 5.0). Infarct (TTZ-negative) and risk (TTZ-stained) zones were determined by a computerized planimetric technique (Minichromax; BioLab, Australia) and the ratio (infarct size/risk zone) was used as the index of myocardial infarction.

Measurement of ECG and the arrhythmia scoring system

ECG was continuously monitored and recorded from standard lead II throughout the experiment with a computerized program (Power Lab). To enable quantitative comparison, a scoring system modified from that used in previous studies (Curtis & Walker 1988; Wong et al 1990) was adopted. The details of the scoring system have been described in a previous study (Yu et al 2001).

Statistical analysis

One-way analysis of variance was adopted to determine the effects of the drugs among different groups while the unpaired Student's *t*-test was employed to determine the effects of the drugs at different concentrations. $P < 0.05$ was considered significant.

Results

Effects of tetrandrine and RST extract in DOCA-salt hypertensive rats

Body weight

The body weight increased in all groups during the experimental course. However, the increase was significantly less in the groups receiving DOCA-salt treatment than that in the normal control group from the 5th week after the left-nephrectomy operation. As shown in Figure 1, in the 19th week after operation, the mean value of body weight in the DOCA-control group was 493 ± 21 g, which was significantly less than that of the normal control group ($74 \pm 3\%$ of the normal control). This is in agreement with previous observations (Balasubramaniam et al 1995; Xu & Rao 1995). Treatment with tetrandrine or RST 50 or 150 mg kg^{-1} for 10 weeks restored the mean body weight towards normal ($87 \pm 3\%$, $86 \pm 2\%$ and $90 \pm 4\%$ of the value in normal controls, respectively). However, only the group treated with RST 150 mg kg^{-1} showed a growth significantly greater than that in DOCA-salt control rats (Figure 1B). The effect of treatment with tetrandrine is in agreement with that shown in a previous study (Xu & Rao 1995).

Wet weight of the heart and right kidney

DOCA-treatment very significantly increased the weight of both the whole heart and left ventricle (LV) in terms of absolute value and percentage value over body weight, and treatment with either RST extract or tetrandrine significantly reduced the increases almost to the level of the controls (Table 1). The effects of DOCA-treatment, with and without tetrandrine, were similar to those observed in a previous study (Xu & Rao 1995). The right ventricle and the ventricular septum also exhibited increases in terms of absolute weight and the normalized weight over the body weight with DOCA-salt treatment. However, neither treatment with the RST extract nor tetrandrine restored the values to control levels. The results indicate that DOCA-salt treatment induced ventricular hypertrophy, especially in the left heart, and that the extract and tetrandrine reduced this hypertrophy.

After left nephrectomy, DOCA-salt treatment also increased the right kidney weight, but this was not attenuated by treatment with either RST extract or tetrandrine.

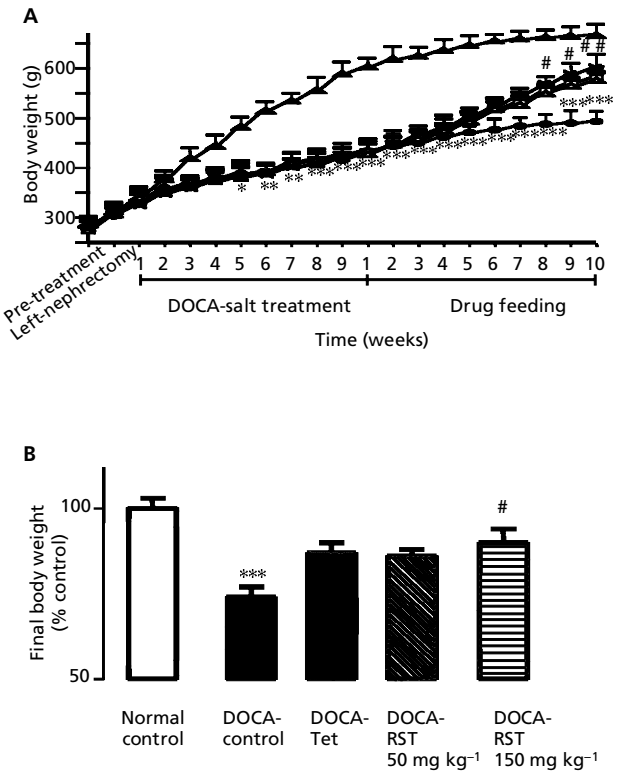

Figure 1 Effects of tetrandrine (Tet) or RST extract on body weight in DOCA-salt hypertensive rats. A. Time-dependent changes in rat body weight during the 10-week DOCA-salt treatment period and 10-week drug-feeding period. ▲, Normal control; ●, DOCA-control; □, DOCA-salt rats fed tetrandine 50 mg kg^{-1} per day (DOCA-Tet); △, DOCA-salt rats fed RST extract (DOCA-RST) 50 mg kg^{-1}; *, DOCA-RST 150 mg kg^{-1}. B. Group results showing changes in body weight at the end of drug treatment. All data are expressed as means ± s.e.m., n = 5 in Normal control and Tet group, n = 6 in other three groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs Normal control; #$P < 0.05$, ##$P < 0.01$ vs DOCA-control.

Systolic arterial blood pressure

Figure 2A shows typical recordings of systolic arterial blood pressure (SAP) with tail-cuff measurement in conscious rats after various treatments. Measurements taken over the whole time course (Figure 2B) show that DOCA-salt treatment induced a sudden and highly significant elevation of SAP in the 3rd week, after which the SAP kept increasing at a slower rate until the 10th week of treatment. The increase was 193 ± 9% of the normal control. Similar increases had been observed in previous studies (Meggs et al 1992; Balasubramaniam et al 1995)

Administration of the RST extract or tetrandrine gradually restored the SAP towards normal. At the end of the 10-week period of drug treatment, the SAP of the groups treated with RST extract or tetrandrine were restored towards the pre-treatment levels (Figure 2C). The effect of the extract was related to dose (Figure 2B, C). The effect of tetrandrine was in keeping with previous findings (Xu & Rao 1995).

Coronary artery flow

The coronary flow was significantly reduced following DOCA-salt treatment and was restored to normal by treatment with either RST extract or tetrandrine (Figure 3). There was a dose-related response to the RST extract (Figure 3). The effect of tetrandrine on coronary flow in DOCA-salt treated rats was similar to that seen in a previous study (Xu & Rao 1995).

P-V curve and left ventricle compliance

In agreement with the previous findings of Xu & Rao (1995), the LVEDP increased with the LV balloon volume and the curve was shifted to the left in the group subjected to DOCA-salt treatment (Figure 4), indicating greater increase in LV pressure with changes in volume and a decrease in myocardial compliance (Horowitz et al 1986; Amrani et al 1994; Manor et al 1995). In DOCA-salt-

Table 1 Effects of tetrandrine or RST extract on heart hypertrophy in DOCA-salt hypertensive rats.

Parameter	Control (n = 5)	DOCA (n = 6)	Tet (n = 5)	RST-50 (n = 6)	RST-150 (n = 6)
HW (mg)	1466.5 ± 31.0	1964.6 ± 28.0***	1575.2 ± 41.0###	1592.2 ± 33.0###	1540.8 ± 21.0###
LVW (mg)	834.5 ± 22.8	1130.7 ± 31.5***	908.1 ± 17.4###	969.0 ± 24.6###	882.6 ± 16.8###
VSW (mg)	315.1 ± 25.4	418.4 ± 19.1*	371.0 ± 21.9	311.2 ± 38.7	331.8 ± 20.5
RVW (mg)	305.1 ± 12.8	403.8 ± 15.5*	356.4 ± 22.3	319.9 ± 21.6	312.0 ± 33.3
RKW (mg)	2974.5 ± 42.8	4271.3 ± 25.4***	4250.9 ± 38.6***	4202.5 ± 28.9***	4257.9 ± 35.6***
HW/BW (mg g^{-1})	2.20 ± 0.31	3.98 ± 0.19***	2.71 ± 0.25#	2.77 ± 0.12#	2.56 ± 0.36##
LVW/BW (mg g^{-1})	1.25 ± 0.14	2.29 ± 0.11***	1.56 ± 0.21#	1.69 ± 0.12#	1.47 ± 0.16##
VSW/BW (mg g^{-1})	0.47 ± 0.07	0.82 ± 0.09*	0.64 ± 0.08	0.54 ± 0.09	0.55 ± 0.07
RW/BW (mg g^{-1})	0.46 ± 0.08	0.82 ± 0.10*	0.61 ± 0.08	0.56 ± 0.08	0.52 ± 0.07
KW/BW (mg g^{-1})	4.46 ± 0.3	8.66 ± 0.9***	7.30 ± 0.5*	7.31 ± 0.6*	7.09 ± 0.5*

All data are expressed as mean ± s.e.m. BW, body weight; HW, wet weight of whole heart; LVW, wet weight of left ventricle; VSW, wet weight of ventricular septa; RVW, wet weight of right ventricle; RKW, wet weight of right kidney; DOCA, DOCA-salt rats without any drug feeding; Tet, DOCA-salt rats with feeding of tetrandrine 50 mg kg^{-1} per day; RST-50 and RST-150, DOCA-salt rats fed with RST extract 50 mg kg^{-1} per day and 150 mg kg^{-1} per day, respectively. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs Normal control; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, vs DOCA control.

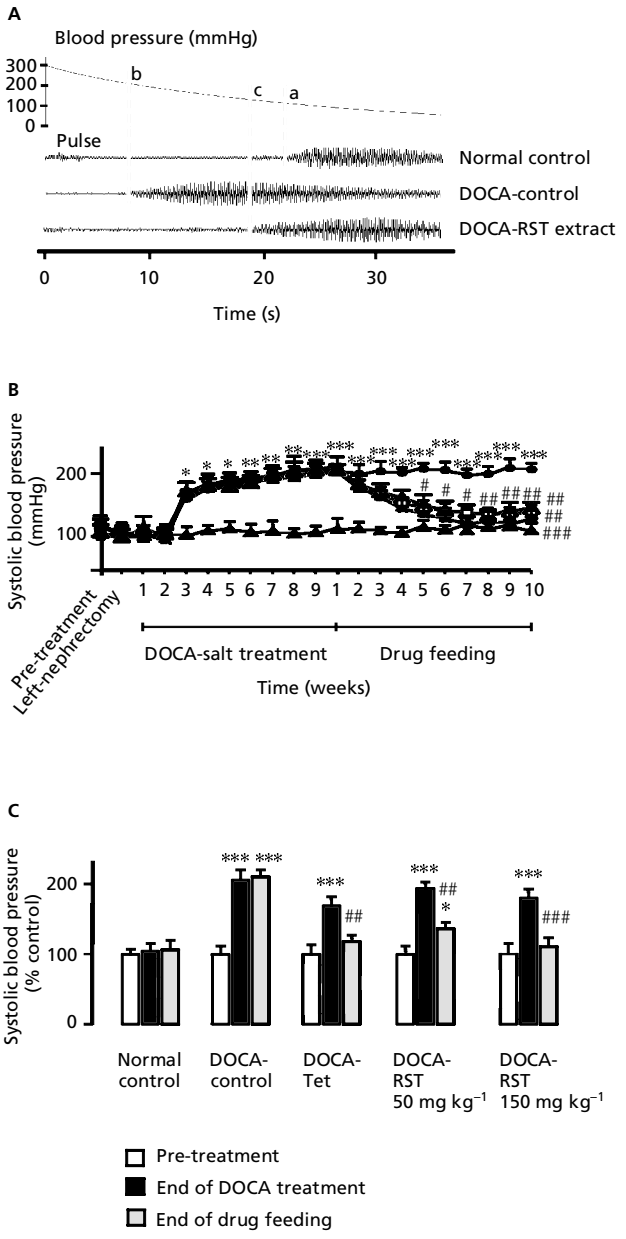

Figure 2 Effects of tetrandrine (Tet) or RST extract on arterial systolic blood pressure in DOCA-salt hypertensive rats. A. Representative traces of the tail-cuff recording for arterial systolic blood pressure measurement. B. Time-dependent changes in the exact values of rat systolic blood pressure during the 10-week DOCA-salt treatment period and 10-week drug-feeding period. ▲, Normal control; ●, DOCA-control; □, DOCA-salt rats fed tetrandine 50 mg kg^{-1} per day (DOCA-Tet); △, DOCA-salt rats fed RST extract (DOCA-RST) 50 mg kg^{-1}; ∗, DOCA-RST 150 mg kg^{-1}. C. Group results as percentage changes of systolic pressure at the end of drug treatment period. The pre-treatment value was 100%. All data are expressed as means ± s.e.m., n = 5 in Normal control and Tet group, n = 6 in the other three groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs Normal control; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$, vs DOCA control.

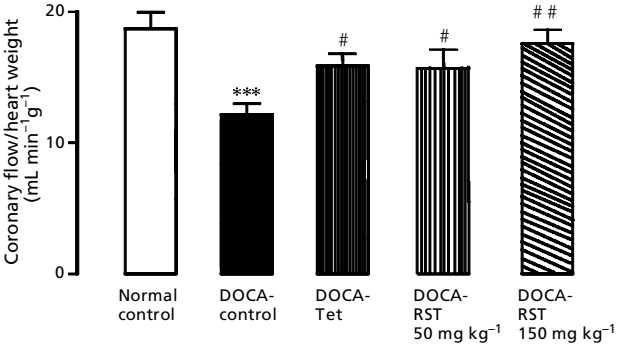

Figure 3 Effects of tetrandrine (Tet) and RST extract on coronary artery flow of isolated perfused hearts from DOCA-salt hypertensive rats. All data are expressed as means ± s.e.m., n = 5 in Normal control and Tet group, n = 6 in each of the other three groups. $***P < 0.001$ vs Normal control; $\#P < 0.05$, $\#\#P < 0.01$ vs DOCA control.

treated groups fed with tetrandrine or the RST extract, the increases in pressure with volume changes were significantly attenuated; the effect of the RST extract was related to the dose (Figure 4).

Effects of tetrandrine, RST extract and verapamil in anaesthetized rats subjected to coronary artery occlusion

Myocardial infarct

After the coronary artery was ligated and released, myocardial infarct occurred (Figure 5). The infarct size was significantly reduced from control (34.5 ± 1.1%) to 19.0 ± 0.9%, 16.5 ± 1.6% and 26.1 ± 1.2% in the groups treated with 2 mg kg^{-1} tetrandrine, 2 mg kg^{-1} RST extract and 25 μg kg^{-1} verapamil, respectively. The effect of the RST extract was similar to that of tetrandrine (Figure 5). The result was the same as in an in-vitro preparation (Yu et al 2001).

Cardiac arrhythmia

In agreement with previous findings in-vitro (Yu et al 1999), ventricular arrhythmias occurred mainly in the first 10 min into reperfusion. The arrhythmia was significantly attenuated by tetrandrine, RST extract or verapamil (Figure 6). The effect of the RST extract was comparable with that of tetrandrine. It is interesting to note that the effect of the extract was also comparable with that of 25 μg kg^{-1} verapamil (Figure 6), the infarct sparing effect of which was significantly less than that of the RST extract (Figure 5). The effects of the RST extract and tetrandrine were the same as in an in-vitro isolated perfused heart preparation (Yu et al 2001).

Discussion

We found that chronic treatment with RST extract not only attenuated the development of hypertension, but also reduced hypertrophy and restored the compliance and

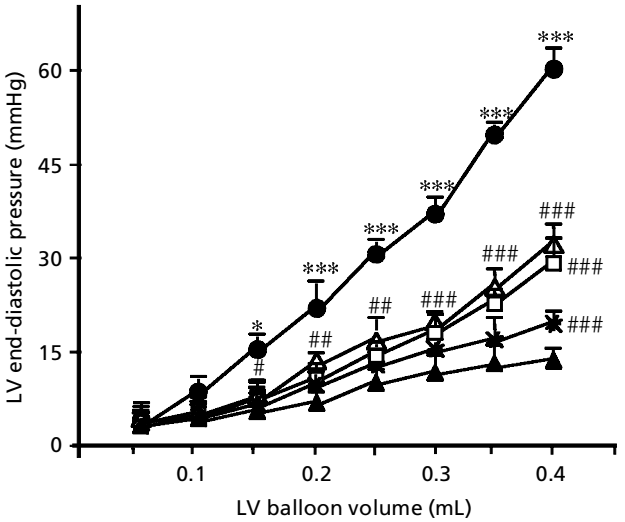

Figure 4 Effects of tetrandrine (Tet) and RST extract on the change in end-diastolic pressure induced by volume changes of left ventricle in perfused heart isolated from DOCA-salt hypertensive rats. ▲, Normal control; ●, DOCA-control; □, DOCA-salt rats fed tetrandine 50 mg kg^{-1} per day (DOCA-Tet); △, DOCA-salt rats fed RST extract (DOCA-RST) 50 mg kg^{-1}; ✱, DOCA-RST 150 mg kg^{-1}. All data were expressed as means ± s.e.m., n = 5 in Normal control and Tet group, n = 6 in the other three groups. $*P < 0.05$, $***P < 0.001$ vs Normal control; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs DOCA-control.

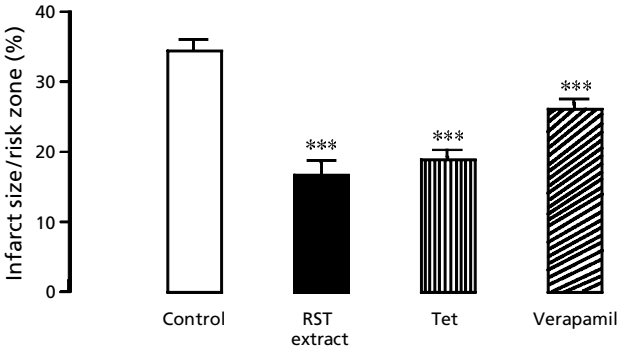

Figure 5 Effects of tetrandrine (Tet), RST extract and verapamil on infarct induced by regional myocardial ischaemia/reperfusion in the anaesthetized open-chest rat. The dose was 25 μg kg^{-1} for verapamil and 2 mg kg^{-1} for Tet and RST extract. Values are presented as means ± s.e.m., n = 8 in Control, n = 9 in treatment groups. $***P < 0.001$ vs Control group.

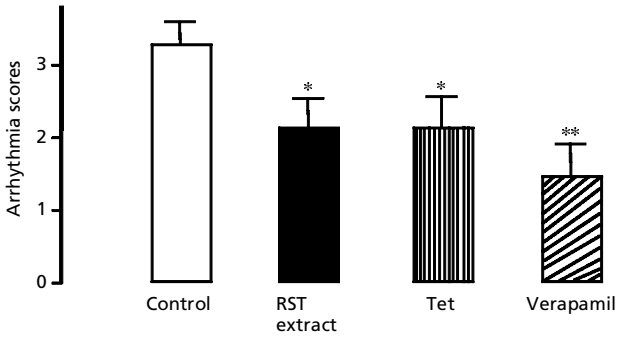

Figure 6 Anti-arrhythmic effects of tetrandrine (Tet), RST extract and verapamil during the first 10 min of reperfusion following regional ischaemia in the anaesthetized rat. The dose was 25 μg kg^{-1} for verapamil and 2 mg kg^{-1} for Tet or RST extract. Values are presented as means ± s.e.m., n = 8 or 9 for all groups. $*P < 0.05$, $**P < 0.01$ vs Control group.

coronary flow of the heart in the DOCA-salt hypertensive rat. In addition, the extract had anti-arrhythmic and infarct-sparing effects in the anaesthetized rat subjected to myocardial ischaemia and reperfusion. The effects were similar to those of verapamil or tetrandrine. The observations indicate that the extract had antihypertensive, anti-arrhythmic and cardioprotective effects in-vivo as do Ca^{2+}-channel antagonists and tetrandrine. The results also indicate that, like Ca^{2+}-channel antagonists, the extract may be useful for the treatment of cardiovascular diseases such as hypertension and ischaemic heart disease.

It is interesting to note that the RST extract produced effects believed to arise from antagonistic actions on the calcium channel in-vivo, as shown in the present study, and in-vitro, as observed previously (Wu et al 2001; Yu et al 2001). This suggests that the components responsible for the action are still present after digestion and absorption in the gastrointestinal tract and transportation in the blood. Further study on the pharmacokinetics of RST is needed.

In previous studies, we showed that an RST extract containing only 9% tetrandrine produced similar (or even more potent) effects to the same weight of tetrandrine alone in the isolated ventricular myocyte or the isolated perfused heart (Wu et al 2001; Yu et al 2001). In this study, we also observed that the RST extract containing only 7% tetrandrine produced the same effects, at a similar potency, as tetrandrine alone. The observations in these studies suggest that tetrandrine may not be the only component responsible for the action of the extract and that interaction between different active components may be more important in contributing to the effect of the extract. Alternatively, most effect in the RST extract may come from something other than tetrandrine. Further study is warranted.

In our experiments with the DOCA-salt hypertensive rat, we used two doses of the RST extract, 50 and 150 mg kg^{-1}. There was an apparent dose-related response in most parameters studied, although only effects on systolic blood pressure and LV end diastolic pressure/volume were of statistical significance. To determine dose-related responses, a greater dose range is needed.

Tetrandrine is the best-characterized Ca^{2+}-entry blocker of plant origin (Sutter & Wang 1993; Wang & Lemos 1995; Takemura et al 1996). However, it expresses lesser selectivity of a Ca^{2+}-entry blocking action (Dong & Yao 1991; Anselmi et al 1994). On the other hand, tetrandrine has been shown to have effects other than inhibition of Ca^{2+}. These effects include interaction with nitric oxide

(Kwan et al 1999) and inhibition of adrenoceptors (Kwan et al 1996). In addition, tetrandrine has wide cardiovascular effects such as hypotensive, cardiodepressant and vasorelaxing actions (Dong and Yao 1991; Anselmi et al 1994). Since the 1950s, tetrandrine has been used in China as an antihypertensive medicine (Gao et al 1965). In the USA, tetrandrine underwent a phase I clinical trial as an anti-tumour medicine (DeConti et al 1975). Further studies are also needed to determine whether the RST extract has similar actions.

Conclusion

We have shown that the RST extract has cardiovascular protection including antihypertensive/anti-hypertrophy actions in the DOCA-salt hypertensive rat and anti-ischaemia/reperfusion-induced myocardial infarct and arrhythmic effects in-vivo. The observations suggest that the RST extract may be a useful agent for the treatment of cardiovascular diseases. Since the RST extract has a similar potency to its main active component, tetrandrine (at the same weight), the action of the extract may not be due solely to tetrandrine.

References

Amrani, F. C. E., Cheaw, S. L., Chevalier, B., Paolaggi, F., Jouquey, S., Hamon, G., Swynghedauw, B. (1994) Regression of left ventricular hypertrophy by converting enzyme inhibition in 12–15-month-old spontaneously hypertensive rats: effects on coronary resistance and ventricular compliance in normoxia and anoxia. *J. Cardiovasc. Pharmacol.* **23**: 155–165

Anselmi, E., Gomez-Lobo, D. M., Blazquez, M. A., Zafra-Polo, M. C., D'Ocon, M. P. (1994) Influence of the absolute configuration on the vascular effects of tetrandrine and isotetrandrine in rat aorta. *Pharmazie* **49**: 440–443

Balasubramaniam, G., Lee, H. S., Mah, S. C. (1995) 5-Carboxamidotryptamine attenuates the development of deoxycorticosterone acetate-salt hypertension in rats. *Eur. J. Pharmacol.* **276**: 183–190

Bianciotti, L. G., De-Bold, A. J. (2000) Effect of selective ET(A) receptor blockade on natriuretic peptide gene expression in DOCA-salt hypertension. *Am. J. Physiol.* **79**: H93–H101

Curtis, M. J., Walker, M. J. A. (1988) Quantification of arrhythmias using scoring system: an examination of seven scores in an in vivo model of regional myocardial ischaemia. *Cardiovasc. Res.* **22**: 656–665

DeConti, R. C., Muggia, F., Cummings, F. J., Calabresi, P., Creasey, W. A. (1975) Clinical and pharmacological studies with d-tetrandrine. *Proc. Am. Assoc. Cancer Res. Am. Soc. Clin. Oncol.* **16:** 96

Dong, H. Y., Yao, C. (1991) Clinical study of isotetrandrine on hypertensive patients. *Clin. J. Integr. Trad. West. Med.* **11**: 87–90

Felix, J. P., King, F., Shevell, J. L., Garcia, M. L., Kaczorowski, G. J., Bick, I. R. C. (1992) Bisbenzylisoquinoline analogs of tetrandrine block L-type calcium channels: evidence for interaction at the diltiazem-binding site. *Biochemistry* **31**: 11793–11800

Gao, Y., Chang, M. Y., Mao, H. Y., Chao, H. Y., Chen, D. H. (1965) Treatment of hypertension (270 cases) and hypertensive crisis with tetrandrine. *Chin. J. Intern. Med.* **13**: 504–507

Horowitz, M., Shimoni, Y., Parnes, S., Gotsman, M. S., Hasin, Y. (1986) Heat acclimation: cardiac performance of isolated rat heart. *J. Appl. Physiol.* **60**: 9–13

Kwan, C. Y. (1994) Plant-derived drugs acting on cellular Ca^{2+} mobilization in vascular smooth muscle: tetramethylpyrazine and tetrandrine. *Stem Cells* **12**: 64–67

Kwan, C. Y., Wang, Z. L. (1993) Tetrandrine: a vasodilator of medicinal herb origin with a novel contractile effect on dog saphenous vein. *Eur. J. Pharmacol.* **238**: 431–434

Kwan, C. Y., Chen, Y. Y., Ma, F. M., Daniel, E. E., Hui, S. C. (1996) Tetrandrine, a calcium antagonist of Chinese herb origin, interacts with vascular muscle alpha 1-adrenoceptor. *Life Sci.* **59**: 359–364

Kwan, C. Y., Ma, F. M., Hui, S. C. (1999) Inhibition of endothelium-dependent vascular relaxation by tetrandrine. *Life Sci.* **64**: 2391–2400

Leung, Y. M., Berdik, M., Kwan, C. Y., Loh, T. T. (1996) Effects of tetrandrine and closely related bis-benzylisoquinoline compounds on cytosolic calcium: a structure-activity study. *Clin. Exp. Pharmacol. Physiol.* **23**: 653–659

Liu, Q. Y., Li, B., Gan, J. M., Kappinski, E., Pang, P. K. T. (1995) Tetrandrine, a Ca^{2+} antagonist: effects and mechanisms of action in vascular smooth muscle cells. *J. Pharmacol. Exp. Ther.* **273**: 32–39

Manor, D., Williams, S., Ator, R., Bryant, K., Scheel, K. W. (1995) Left ventricular mechanics in arrested dog heart: effects of ventricular interaction and vascular volumes. *Am. J. Physiol.* **268**: H2125–H2132

Meggs, L. G., Huang, H., Tillotson, J., Capasso, J. M., Anversa, P. (1992) Re-expression of α skeletal actin and regulation of α1 adrenoceptor signalling in DOCA-salt hypertension in rats. *Cardiovasc. Res.* **26**: 878–885

Muthalif, M. M., Benter, I. F., Khandekar, Z., Gaber, L., Estes, A., Malik, S., Parmentier, J. H., Manne, V., Malik, K. U. (2000) Contribution of Ras GTPase/MAP kinase and cytochrome P450 metabolites to deoxycorticosterone-salt-induced hypertension. *Hypertension* **35**: 457–463

Shen, Y. C., Chen, C. F., Wang, S. Y., Sung, Y. J. (1999) Impediment to calcium influx and reactive oxygen production accounts for the inhibition of neutrophil mac-1 up-regulation and adhesion by tetrandrine. *Mol. Pharmacol.* **55**: 186–193

Solomon, S. B., Nikolic, S. D., Glantz, S. A., Yellin, E. L. (1998) Left ventricular diastolic function of remodeled myocardium in dogs with pacing-induced heart failure. *Am. J. Physiol.* **274**: H945–H954

Sutter, M. C., Wang, Y. X. (1993) Recent cardiovascular drugs from Chinese medicinal plants. *Cardiovasc. Res.* **27**: 1891–1901

Takemura, H., Imoto, K., Ohshika, H., Kwan, C. Y. (1996) Tetrandrine as a calcium antagonist. *Clin. Exp. Pharmacol. Physiol.* **23**: 751–753

Wang, G., Lemos, J. R. (1995) Tetrandrine: a new ligand to block voltage-dependent Ca^{2+} and Ca^{2+}-activated K^+ channels. *Life. Sci.* **56**: 295–306

Wang, H. X., Kwan, C. Y., Wong, T. M. (1997) Tetrandrine inhibits electrically induced $[Ca^{2+}]_i$ transient in the isolated single rat cardiomyocyte. *Eur. J. Pharmacol.* **319**: 115–122

Wong, T. M., Lee, A. Y. S., Tai, K. K. (1990) Effects of drugs interacting with opioid receptors during normal perfusion or ischaemia and reperfusion in the isolated rat heart – an attempt to identify cardiac opioid receptor subtype(s) involved in arrhythmogenesis. *J. Mol. Cell. Cardiol.* **22**: 1167–1175

Wu, S., Yu, X. C., Shan, J., Wong, T. M., Chen, C. F., Pang, K. T. (2001) Cardiac effects of the extract and active components of radix stephaniae tetrandrae I. Electrically-induced

intracellular calcium transient and protein release during the calcium paradox. *Life Sci.* **68**: 2853–2861

Xu, Y., Rao, M. R. (1995) Effects of tetrandrine on left ventricle hypertrophy in deoxycorticosterone acetate-salt hypertensive rats. *Eur. J. Pharmacol.* **278**: 1–7

Yu, X. C., Wang, H. X., Pei, J. M., Wong, T. M. (1999) Anti-arrhythmic effect of kappa-opioid receptor stimulation in the perfused rat heart: involvement of a cAMP-dependent pathway. *J. Mol. Cell. Cardiol.* **31**: 1809–1819

Yu, X. C., Wu, S., Wang, G. Y., Shan, J., Wong, T. M., Chen, C. F., Pang, K. T. (2001) Cardiac effects of the extract and active components of radix stephaniae tetrandrae II. Myocardial infarct, arrhythmias, coronary arterial flow and heart rate in the isolated perfused rat heart. *Life Sci.* **68**: 2863–2872