Effects of tetrandrine on smooth muscle contraction induced by mediators in pulmonary hypertension[1]

WANG Huai-Liang[2], ZHANG Xin-Hua, CHANG Tian-Hui

Department of Clinical Pharmacology, China Medical University, Shenyang 110001, China

ABSTRACT

AIM: In attempt to characterize tetrandrine on pulmonary hypertension, biological activities induced by a range of mediators implicated in the pathogenesis of pulmonary hypertension were investigated. **METHODS:** Pulmonary artery rings and tracheal segments were contracted with couples of bioactive substances in which a series experiments including effects of tetrandrine on calcium agonist, endothelin, thromboxane A_2, angiotensin II, neuropeptide Y, histamine, 5-methyl furmethide were performed, the influences of tetrandrine in the concentration of 1 to 30 µmol/L were investigated. **RESULTS**: Tetrandrine inhibited calcium agonist BayK8644, endothelin-1 and thromboxane A_2 mimetic U46619, angiotensin II- and neuropeptide Y-induced contractile responses with depression of the maximal contraction of pulmonary artery rings in a varying extent. Tetrandrine inhibited leukotriene E_4-induced concentration-response curve in a competitive antagonist manner with a pK_B of (5.29 ± 0.11) without any influence leukotriene C_4, leukotriene D_4, histamine, and 5-methylfurmethide induced contractile responses of guinea pig trachea. **CONCLUSION**: Tetrandrine may produce multiple pharmacological effects against calcium channel antagonist, U46619, endothelin-1, angiotension II, and neuropeptide Y induced vasoconstriction in rat pulmonary arteries in varying extent and inhibition of leukotriene E_4 rather than C_4, D_4, histamine, and 5-methyl furmethide induced contractile responses on rat tracheal segments. These pharmacological characteristics are considered to contribute to its antihypertensive action during pulmonary hypertension.

INTRODUCTION

Tetrandrine (TET) has long been used in traditional Chinese medicine as antihypertensive and anti-asthmatic agent[1]. Recently we found that TET displayed a pref-erential vasodilator effect on the pulmonary circulation during acute hypoxic pulmonary hypertension[2]. TET inhibited pulmonary vascular constriction and remodeling of monocrotaline (MCT)-induced chronic "inflammatory" pulmonary hypertension in the rat[3,4]. TET also inhibited growth factors-induced proliferation and mitogenesis of cultured pulmonary smooth muscle cells (PASMC)[5]. It is well known that pulmonary hypertension comprised two major components, *ie*, pulmonary vascular constriction and remodeling. Although the exact role of pulmonary hypertension is poorly understood, but as the only organ in the body that re-

---

ceives the entire cardiac output, the lung produces, stores and metabolizes couples of vasoactive substances. Several candidates had been taken into consideration in the pathophysiology of pulmonary hypertension, *eg*, Ca^{2+} channels[6], thromboxane A_2[7], endothelin-1[8], leukotrienes, histamine, angiotensin II, and neuropeptide Y[9]. Much evidence suggested that TET produced vasodilator effect was related to calcium channel blockade mechanism[10]. Yet, it is not clear whether this plant alkaloid may have any effect on these possible mediators involved in pulmonary hypertension. In the present work, actions of TET on these possible mediators were investigated to study the possible mechanisms of action of TET.

MATERIALS AND METHODS

Chemicals and solutions Tetrandrine, domethacin, *S*-(-)BayK8644: (*S*[-]-1, 4-Dihydro-2, 6-dimethyl)-3-pyridine carboxylic aid methyl ester) and charbamylcholine chloride (carbachol) histamine hydrochloride, angiotensin II, yohimbine hydrochloride, pargyline hydrochloride; phenoxybenzamine hydrochloride were purchased from Sigma St Louis, MO, USA. Leukotriene C_4, D_4, E_4 from Cascade Biochem Limited; 5-methyl furmethide from H Hadson; U46619 from Cayman; neuropeptide Y was kind gift from Dr GR MARTIN, Glaxo Wellcome. Tetrandrine was dissolved in 0.1 mol/L-HCl and then titrated to pH 5.5 with 0.1 mol/L NaOH as stock solution. U46619 was dissolved in absolute ethanol. Others were dissolved in distilled water.

Preparation of rat pulmonary artery rings Male Sprague Dawley rats from Animal Resource Center of China Medical University (Certificate No: Liaoning-034) weighing 300-350 g were used in this study. The animals were housed in a temperature (22 ± 2) °C and humidity (60 %-70 %) controlled room and allowed free access to standard rat pellet diet and tap water. After killing the animal by a blow to the head, the chest was opened and heparin sulfate (500 U) was injected into the right ventricle to prevent blood coagulation in the pulmonary vasculature. The heart and lungs were removed *en bloc*. The main pulmonary artery was rapidly dissected free and placed in Kreb-Hensleit's solution (composition in mmol/L: NaCl 118.41; $NaHCO_3$ 25.00; KCl 4.75; KH_2PO_4 1.19; $MgSO_4$ 1.19; glucose 11.10 and $CaCl_2$ 2.50). Rings were cut into 2 mm width and mounted on tungsten hooks attached to a tissue holder and a force displacement transducer and sus-

pended in an organ bath containing Kreb Hensleit's solution maintained at 37 °C, gassed with a mixture of 95 % O_2 and 5 % CO_2. The lower hook was attached to a perpex tissue holder and the upper hook was attached to an isometric Grass Futon transducer.

The pulmonary artery rings were treated as described else where, briefly after setting up this tissue, pargylin 500 mmol was added into the buffer to inactive monoamine oxidase (MAO). Resting tension of the artery rings kept at 1 g by three stretches within 30 min with washing out followed each stretch in the interval of 10 min between two stretches. Indomethacin (5 μmol/L) was included in the buffer to block the formation of cyclooxygenase products. Acetylcholine (ACh) 0.3 μmol/L was used to check the function of vascular endothelium to assure all rings used were without endothelium damage. Potassium chloride (KCl) was added into the organ bath with a final concentration of 80 mmol/L to get the maximum contraction which was then used as a reference through the remainder of the experiments. After thorough wash out, paired agonists-induced concentration-effect curves were constructed. Tetrandrine in different concentrations or vehicle were added 1 h before the second curve. Data were expressed as percentage of maximal contraction of KCl.

Preparation of guinea pig trachea rings Male Dunkin-Hartley guinea pigs weighing 300-500 g were killed by cervical dislocation and then the trachea was removed. Tracheal segments were prepared as descried previously and placed in 5-mL organ baths maintained at 37 °C and attached with tungsten hooks to Grass force transducers with a resting tension of 3 g. The tissues were bathed in Kreb Hensleit's solution and gassed with a mixture of 95 % O_2 and 5 % CO_2. Tissues were allowed an equilibration period of 60 min before undergoing experimentation. The bath fluid contained indomethacin (5 μmol/L) to remove the possible influence of cyclooxygenase metabolites[11].

Single cumulative concentration-effect curves were obtained from tracheal segments by successive increases in the bath of the concentration of agonists by the method as described[12]. Contractile response was expressed as a percentage of the response obtainable to a maximally effective concentration of carbachol (30 μmol/L) added to the bath after the concentration-effect curves.

Isolated guinea pig tracheal segments are capable of metabolizing peptide of LTs[11]. Experiments with LTC_4 were conducted in the presence of acivicin (30

µmol), a γ-glutamyl transpeptidase inhibitor incubated with the tissue for 30 min before the starting of the concentration-effect curves eliminating the biological consequence of any conversion of LTC_4 to LTD_4 during study[13]. Experiments with LTD_4 on trachea were performed in the presence of *L*-cysteine (3 mmol/L) to prevent conversion of LTD_4 to LTE_4[14].

Statistics The data was presented as Mean±SD. Statistical significant differences were determined with unpaired *t*-test. *P*<0.05 was considered significant.

RESULTS

Effects of tetrandrine on responsiveness of pulmonary artery rings Addition of tetrandrine (30 µmol/L) *per se* produced no effect on the resting tension of the rings. But TET inhibited Bay K8644, U46619, ET-1, angiotension II, and NPY induced contraction of pulmonary rings in a non-competitive manner. Yet, there exist variation of the inhibition among these mediators.

Compound Bay K8644 induced a concentration dependent contraction of rat pulmonary artery rings with an EC_{50} of 1.5 nmol/L. Tetrandrine 30 µmol/L, rather than 10 µmol/L, significantly depressed Bay K8644 induced maximal contraction from 78.3 %±2.8 % to 38.4 %±2.1 % (*P*<0.05) and the EC_{50} increased from 1.5 nmol/L up to 10 nmol/L, *P*< 0.05) (Fig 1).

As Fig 2 shown that TXA_2 mimetic U46619 caused concentration-dependent contraction of rat pulmonary

Fig 2. Effect of tetrandrine on U46619-induced contraction of rat pulmonary artery. In each case data are average of 3-4 replicate experiments. Mean±SD. KCl=80 mmol/L

artery rings. TET 10 µmol/L significantly depressed U46619 induced concentration-effect curves from 149 %±7 % to 88 %±4 % (*P*<0.05) with a 41.5 % reduction of the maximal contraction and the EC_{50} increased from 2.1 nmol/L to 3 nmol/L.
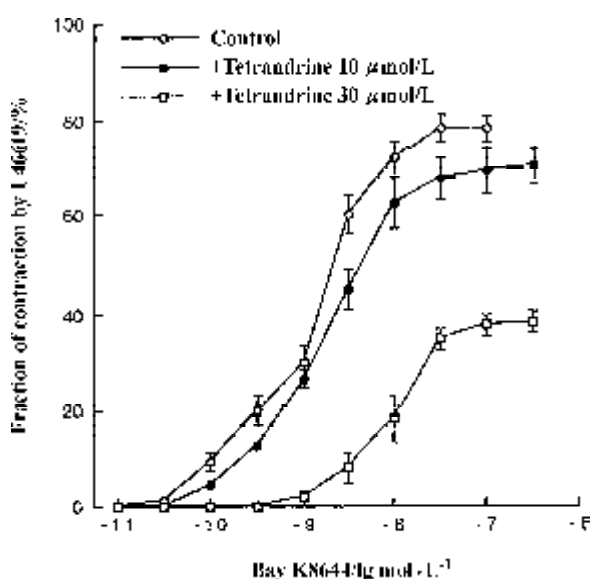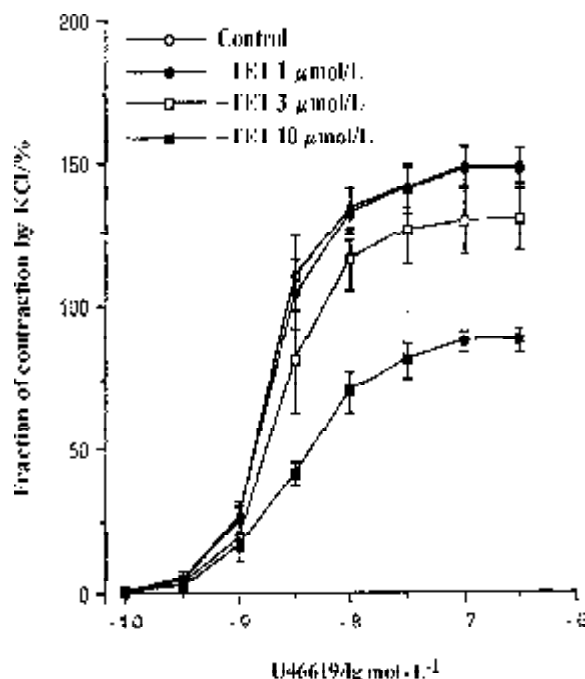
As Fig 3 shown that ET-1 caused a slow onset

Fig 1. Effect of tetrandrine on Bay K8644-induced contraction of rat pulmonary artery rings. In each case data are average of 7-8 replicate experiments. Mean±SD.
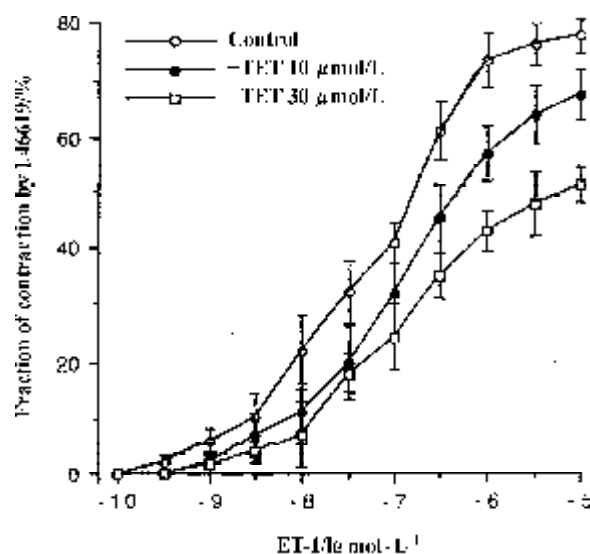

Fig 3. Effect of tetrandrine on endothelin-1-induced contractile response of rat pulmonary artery rings. In each case, data are the average of 3-4 replicate experiments. Mean±SD.

and long lasting contractile effect on the pulmonary artery rings with maximal contraction of 77.6 %±2.9 %. TET inhibited ET-1-induced contraction in a concentration-dependent and a non-competitive manner. In the concentration of 10 and 30 μmol/L TET depressed the maximal contraction from 77.6 %±2.9 % to 67 %± 4 % and 51 %±3 % (*P*< 0.05) respectively with slight, but not significant increase of EC_{50}.

As shown in Fig 4, angiotensin II induced concentration dependent contractile response of rat pulmonary artery rings. Addition of tetrandrine from 1 to 30 μmol/L *per se* produced no effect on the resting tension but in the concentration of 30 μmol/L depressed angiotensin ll concentration-response curve, in which the maximal contraction of angiotensin ll was diminished from 174 %±9 % to 114 %±7 % and the EC_{50} was increased from 10 nmol/L up to 30 nmol/L, *P*< 0.05.
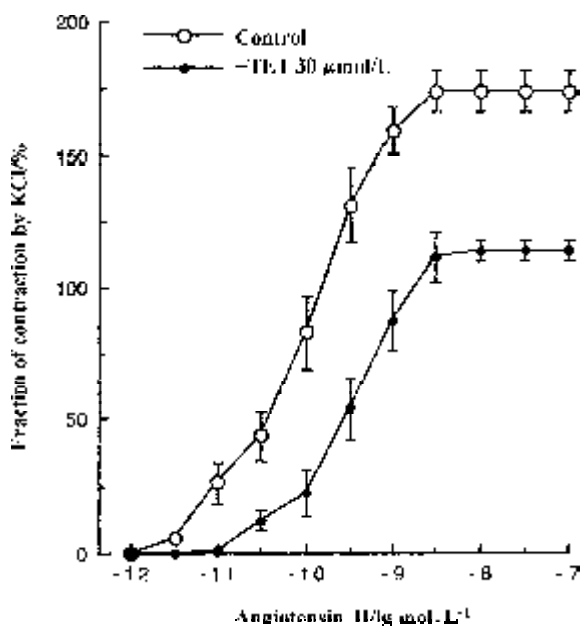

Fig 4. Effect of tetrandrine on angiotensin ll-induced contraction of rat pulmonary artery. In each case data are average of 3-4 replicate concentration-effect curves. KCl=80 mmol/L. Mean±SD.

As shown in Fig 5, the NPY induced concentration-response curves were constructed on the top of angiotensin ll induced contraction, in which the angiotensin ll induced contraction serves as the "base line" of NPY contractile response. TET in the concentration of 1, 3 and 10 μmol/L produced a concentration-dependent inhibition of the "base line" by 7 %, 9 %, and 20 %, respectively. TET also inhibited the contractile
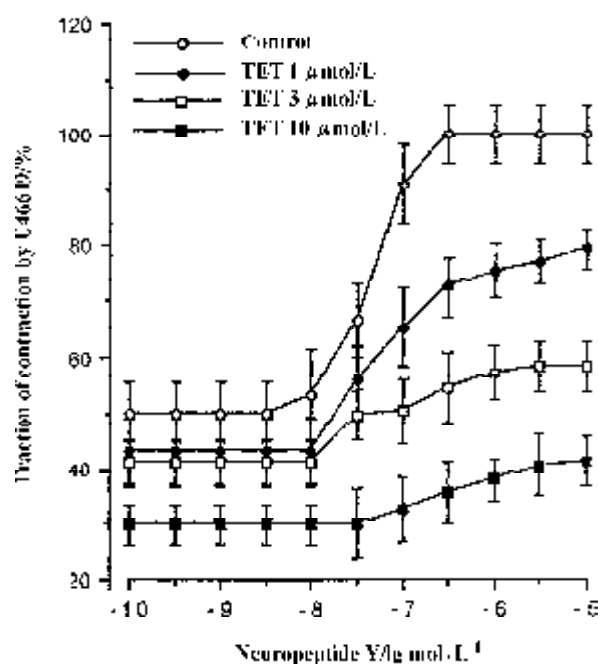

Fig 5. Effect of tetrandrine on neuropeptide Y-induced contraction of rat pulmonary artery rings. In each case data are average of 4 replicate experiments. Mean±SD.

response by NPY from 92 %±4 % to 78 %±4 %, 68 %±5 % and 41 %±4 %, respectively. Obviously the inhibition caused by tetrandrine was non-competitive.

Effects of tetrandrine on responsiveness of guinea-pig trachea Leukotriene C_4, D_4 and E_4 caused concentration dependent contraction of guinea pig tracheal segments. LTC_4- and LTD_4-induced concentration-response curves were not significantly influenced by TET (Fig 6). However, the concentration-effect curve produced by LTE_4 was significantly shifted rightward by tetrandrine. The calculated dissociation constant (pK_B) for tetrandrine against LTE_4 receptors was 5.29±0.11.

Tetrandrine produced no effects on histamine or 5-methylfurmethide induced contractile responses in the guinea pig trachea (data not shown).

DISCUSSION

That tetrandrine as a vasodilator and antihypertensive in the Chinese armamentarium for cardiovascular diseases[4] has raised a lot of interest in the pharmacology of this drug which has been shown to block the L-type voltage operated calcium channels (VOCC) and agonist-induced contraction through putative receptor operated calcium channels ROCC[1]. Not until recently was it revealed that tetrandrine combined with K^+ (in a
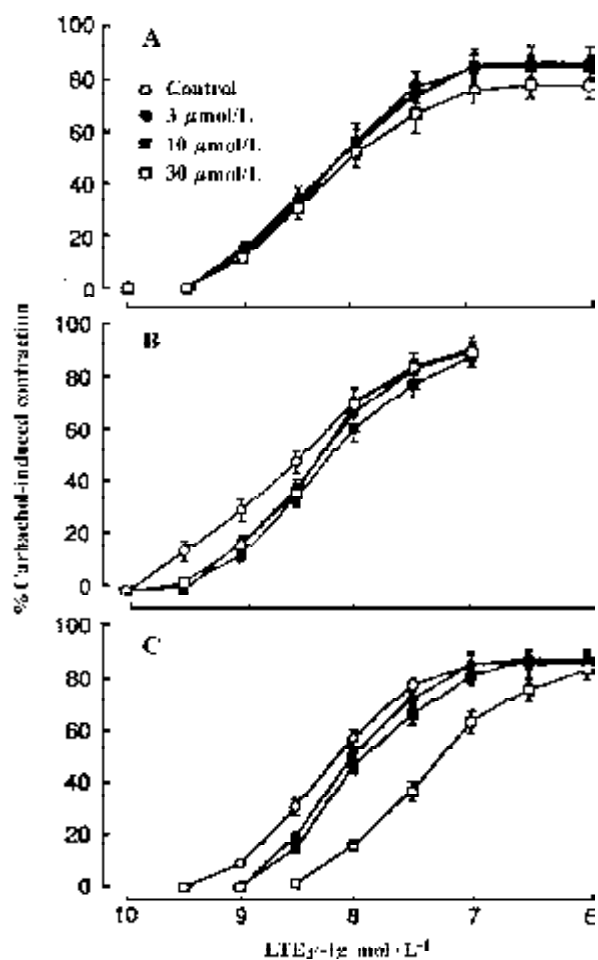
Fig 6. Antagonism by tetrandrine on contractions produced by LTC$_4$ (panel A), LTD$_4$ (panel B) and LTE$_4$ (panel C) in guinea pig trachea. Symbols denote in the absence or presence of tetrandrine at the concentrations shown. In each case data are average of 3-4 replicate experiments. Ordinate: increase in tissue force expressed as a fraction of the challenge with carbachol (30 mmol/L) at end of each concentration-effect curve. Mean±SD.

still unclear relationship, but probably permissively) to promote a neuronal and α_1-adrenoceptor mediated contraction[1].

Changes in interacellular Ca^{2+} concentration [Ca^{2+}]$_i$ are critical in smooth muscle contraction or relaxation and the initiation of other cellular responses. The regulation of [Ca^2]$_i$ involves a complex interaction between Ca^2 entry and extrusion across the plasmalemma and Ca^{2+} release and re-uptake from the sarcoplasmic reticulum. This Ca^{2+} movement is controlled mainly by Ca^{2+} channels. There are several types of voltage-dependent Ca^{2+} channels, including L-type, T-type, N-type and P-type, which differ in structure, function, and pharmacological properties[15]. Voltage-

dependent Ca^{2+} channels on smooth muscle cells are usually of the L-type, whereas the release of intracellular Ca^{2+} is controlled by IP$_3$ receptors on the sarcoplasmaic reticulum[16]. It had been suggested that hypoxia may close oxygen-sensitive K$^+$ channels which leads to smooth muscle depolarization and Ca^{2+} entry, inducing contraction. Functional studies also suggest that Ca^{2+} channels are important in the regulation of pulmonary vascular tone. It was found that Bay K8644, a dihydropyridine Ca^{2+} agonist, potentiated the constrictor response to angiotensin II or PGF$_{2\alpha}$. However, the constrictor effect on hypoxic pulmonary hypertension is greater than on angiotensin II contraction, suggesting that hypoxic pulmonary vasoconstriction is more dependent on extracellular Ca^{2+}[17]. The present study demonstrated that tetrandrine inhibited Bay K 8644 induced contraction of rat pulmonary artery rings in a dose dependent manner. The calcium channel antagonistic activity of tetrandrine on pulmonary artery obtained in this study is in agreed with those findings on other tissues eg guinea pig atrium[18] and canine cardiac Pukinje fibres[19]. It has been shown that tetrandrine inhibits both L-type and T-type calcium channels[20]. It is possible therefore that tetrandrine produced inhibition of Bay K8644 induced contractile response of rat pulmonary artery rings may explain the activity of this drug in preventing or removing hypoxic pulmonary hypertension and that this action might involve both L-type and T-type calcium channels.

Our recent studies showed that tetrandrine had preferred selectivity to improve acute hypoxic pulmonary hypertension in rats and in dogs[2]. It is known that several bio-active mediators are considered to be involved in the pathophysiology of pulmonary hypertension. Results from the present study demonstrated that tetrandrine inhibited Bay K 8644, and also ET-1, U46619, angiotensin II and NPY induced contractile responses of rat pulmonary artery rings in a varying extent. Tetrandrine had no significant effect on histamine, muscarinic receptors for acetylcholine or leukotriene C$_4$, and D$_4$ induced contractile responses, yet it produced a competitive antagonistic activity on LTE$_4$ induced contractile response of guinea pig trachea.

The present study also demonstrated that tetrandrine produced concentration-dependent inhibition of rat pulmonary artery contraction caused by the thromboxane A$_2$ mimetic U46616. It is known that TXA$_2$ and its stable breakdown product TXB$_2$ are pulmonary vasoconstrictors. Some of the vasoconstrictor's

effect is considered to mediate the endothelium dependent contractile response to arachidonic acid and methacholine in rat pulmonary artery *in vitro*. The inhibitory activity of tetrandrine on U46619 induced contractile response of rat pulmonary artery rings would contribute to the ability of this drug to dilated pulmonary vasculature, decrease the pulmonary vascular resistance and decrease pulmonary hypertension. The non-competitive antagonism of tetrandrine against U46619 induced contraction on the pulmonary artery rings might be related with its calcium antagonist activity.

Endothelin-1 (ET-1) possesses important biological and pharmacological properties: it induces a systemic hypertension and bronchoconstriction in the guinea pig, produces a potent and long lasting constriction in vascular and non-vascular isolated organs and appears to have a specific recognition site in smooth muscle cells. In the pulmonary circulation, endothelin-1 increases vascular pressure through its action on pulmonary vascular smooth muscle. In particular, ET-1 has been shown to have vasoconstrictor activity in the pulmonary vascular bed of many species, including the cat, rat, dog, and human[9]. In addition, plasma levels of ET-1 are elevated in patients with pulmonary hypertension. ET-1 may elicit vasoconstriction through calcium channel activation, protein kinase C mediation or arachidonic acid release. Endothelin-1 induced vasoconstriction is dependent on extracellular calcium influx. Barman reported that verapamil (10 μmol/L) inhibited the canine pulmonary vascular response to ET-1[8,21].

A recent study examed the effects of bosentan, an orally active antagonist of endothelin-A and -B, on the development and maintenance by hypoxia (10 % O_2)-induced pulmonary hypertension and vascular remodelling in the rat. Pre-treatment with bosentan (100 mg·kg^{-1}·d^{-1}, 1 gavage/day for 2 d) completely blocked the pulmonary constrictor response to acute hypoxia.

The present study demonstrated that tetrandrine had significant inhibitory effects on endothelin-1 induced contractile response of rat pulmonary artery. This effect might be closely related to its hypotensive effect on pulmonary hypertension. Tetrandrine had been shown to inhibit KCl induced rat pulmonary pressor response. Taken together, these observations suggest that the ability of tetrandrine to inhibit ET-1 induced contraction of rat pulmonary artery rings involves inhibition on calcium channels.

Arachidonic acid is an essential fatty acid and an integral component of different phospholipase pools in the cell membrane. The lipoxygenase metabolites of arachidonic acid LTB_4, LTC_4, LTD_4 and LTE_4 had been reported to have diverse biological activities, including the contractor effects on isolated pulmonary artery rings of guinea pig, pig, isolated rat lungs and human, and the intact pulmonary vascular bed *in vivo*[22]. While there is an agreement that LTs are produced in the lung, it is less certain as to how they affect the pulmonary vasculature particularly in different species. Using more specific agents, Cassin *et al* have demonstrated that LTD_4 in newborn lambs and goats mediates its pressor effects *via* TxA_2 release but has no role in hypoxic pulmonary hypertension. In contrast, in the rat, LTC_4 and LTE_4 have been shown to cause sustained pulmonary pressor responses independent of cyclooxygenase products, albumin or blood[22]. So in this study we used guinea pig tracheal segments to evaluate whether tetrandrine had any effects against LTs.

Our previous experiments indicated that the bronchial alveolar lavage fluid (BALF) from acute hypoxia ventilated rats triggered the contraction of guinea pig ileum reflecting SRS-A like substances involved in hypoxic response of rats[3]. The present study demonstrated that tetrandrine produced a competitive antagonism on leukotriene E_4, rather than leukotriene C_4 and leukotriene D_4 induced contractile responses of guinea pig tracheal segment. The antagonistic activity of tetrandrine on leukotriene E_4 might be related with its inhibitory activity on BALF induced contraction of the ileum, in which its calcium antagonist activity is possibly involved. It is possible therefore that in intact animals tetrandrine may inhibit hypoxia induced responses including tracheal smooth muscle as well as pulmonary vessels as well.

In conclusion, the present results revealed the possibility of multiple mechanism involved in tetrandrine induced pharmacological effect against pulmonary hypertension, *eg*, calcium channel blockade and couples of bioactive mediators related pulmonary vascular contractility in a varying extent.

REFERENCES

1   Achike FI, Kwan CY. Characterization of a novel tetrandrine-induced contraction in rat tail artery. Acta Pharmacol Sin 2002; 23: 698-704.

2   Wang HL, Liu KJ, Zhang XH, Jin X, Xing J. Effects of tetrandrine on acute hypoxic pulmonary hypertension. Chin J Pharmacol Toxicol 1994; 8: 246-9.

3   Wang HL, Liu KJ, Zhang XH, Jin X, Xing J. Effect of tetrandrine on monocrotaline-induced pulmonary hyperten-

sion in the rat. Acta Pharmacol Sin 1997: 18: 400-4.

4　Wang HL, Liu KJ, Zhang XH, Jin X, Xing J. Tetrandrine inhibits chronic "inflammatory" pulmonary hypertension in rats. Drug Dev Res USA 1997; 39: 159-60.

5　Wang HL, Kilfeather SA, Page CP, Martin GR, Pogson CI. Effect of tetrandrine on growth factor-induced DNA synthesis and proliferation of rat pulmonary arterial smooth cells. J Pulmonary Pharmacol Ther 2000; 13: 53-60.

6　Barnes PJ, Baraniuk J, Belvisi MG. Neuropeptides in the respiratory tract. Am Rev Respir Dis 1991; 144: 1187-98.

7　Ono S, Voelkel NF. PAF antagonists inhibit monocrotaline-induced lung injury and pulmonary hypertension. J Appl Physiol 1991; 71: 2483-92.

8　Jin XR, Fan M, Wang ZQ, Su YC, Yang GT, Hu HB, *et al*. Strain difference in pulmonary vascular responsiveness to hypoxia in rats. J Tongji Med Univ 1990; 10: 134-40.

9　Lippton HL, Hamari C, Feng CJ, Chen BQ, Summer WR. Peptidergic regulation of the lung circulation. Crit Care Report 1990; 1: 266-74.

10　Soifer SJ, Schreiber MD, Heymann MA. Leukotriene antagonists attenuate thromboxane-induced pulmonary hypertension. Pediatr Res 1990; 26: 83-7.

11　Krell DR, Osborn R, Vickery L, Falcone K, Gleason J. Contraction of isolated airway smooth muscle by synthetic leukotrienes C4 and D4. Prostaglandins 1981; 22: 387-409.

12　Van Rossum JM. Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch Int Pharmacodyn Ther 1963; 143: 229-330.

13　Ibe BO, Campbell WD. Synthesis and metabolism of leukotrienes by human endothelial cells, influence on prostacyclin release. Biochem Biophys Acta 1988; 960: 309-21.

14　Boot JR, Bond AR, Gooderham R, O'Brien A, Parsons M, Thomas KH. The pharmacological evaluation of LY170680, a novel leukotriene D4 and E4 antagonist in guinea-pig. Br J Pharmacol 1989; 98: 259-67.

15　Tsiew RW, Ellinor PT, Horne WA. Molecular diversity of voltage-dependent Ca^{2+} channels. Trends Pharmacol Sci 1991; 12: 349-53.

16　Marks AR. Calcium channels expressed in vascular smooth muscle. Circulation 1992; 86 Suppl III: 61-7.

17　McMurtry IF. Humoral control in abnormal pulmonary circulation. Bergosfky EH, editor. Philadelphia: Churchill Livingstone: 1986. p 83-125,

18　Fang DC, Xia GJ, Qu L, Jiang MX. Studies on the calcium antagonist action of tetrandrine . I. Effects of tetrandrine on the isolated guinea pig atrium. Acta Acad Med Wuhan 1981; 1: 46-50.

19　Wang G, Zong XG, Fang DC, Jiang MX, Lu FH. Effects of tetrandrine on the slow inward currents in canine cardiac Pukinje fibres. Acta Pharm Sin 1988; 23: 646-50.

20　Felix JP, King VF, Shevell JL, Garcia ML, Kaczorowski GJ, Bick IRC, *et al*. Bis-benzylisoquinoline analogs of tetrandrine block L-type calcium channels: evidence for interaction at the diltiazem-binding site. Biochemistry 1992; 31: 11793-800.

21　Shimamoto Y, Shimamoto H, Kwan CY, Danniel EE. Differential effects of putative protein kinase C inhibitors on contraction of rat aortic smooth muscle. Am J Physiol 1993; 264: H1300-6.

22　Voelkel NF, Stenmark KR, Reeves JT, Mathias MM, Murphy RC. Actions of lipoxygenase metabolites in isolated rat lungs. J Appl Physiol 1984; 57: 860-7.