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

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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 A₂, 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 A₂ 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 E₄-induced concentration-response curve in a competitive antagonist manner with a pKᵦ of (5.29 ± 0.11) without any influence leukotriene C₄, leukotriene D₄, histamine, and 5-methylfurthide induced contractile responses of guinea pig trachea. CONCLUSION: Tetrandrine may produce multiple pharmacological effects against calcium channel antagonist, U46619, endothelin-1, angiotensin II, and neuropeptide Y-induced vasoconstriction in rat pulmonary arteries in varying extent and inhibition of leukotriene E₄ rather than C₄, D₄, 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 preferential 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, e.g. Ca\textsuperscript{2+} channels\textsuperscript{[6]}, thromboxane A\textsubscript{2}\textsuperscript{[7]}, endothelin-1\textsuperscript{[8]}, leukotrienes, histamine, angiotensin II, and neuropeptide Y\textsuperscript{[9]}. Much evidence suggested that TET produced vasodilator effect was related to calcium channel blockade mechanism\textsuperscript{[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, pargline hydrochloride; phenoxybenzamine hydrochloride, angiotensin II, and neuropeptide Y were dissolved in absolute ethanol. Others were dissolved in 0.1mol/L NaOH as stock solution. U46619 was dissolved in 0.1 mol/L-HCl and then titrated to pH 5.5 with NaOH to get the maximum contraction which was expressed as a percentage of the response obtained from tracheal segments by successive 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 described 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\textsubscript{2} and 5 % CO\textsubscript{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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[11]}. Experiments with LTC\textsubscript{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 LTC₄ to LTD₄ during study[13]. Experiments with LTD₄ on trachea were performed in the presence of L-cysteine (3 mmol/L) to prevent conversion of LTD₄ to LTE₁[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 EC₅₀ 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 EC₅₀ increased from 1.5 nmol/L up to 10 nmol/L, P< 0.05) (Fig 1).

As Fig 2 shown that TXA₂ mimetic U46619 caused concentration-dependent contraction of rat pulmonary 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 EC₅₀ increased from 2.1 nmol/L to 3 nmol/L.

As Fig 3 shown that ET-1 caused a slow onset
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 EC50.

As shown in Fig 4, angiotensin II induced concentration dependent contractile response of rat pulmonary artery rings. Addition of tetrangrine 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 II concentration-response curve, in which the maximal contraction of angiotensin II was diminished from 174 %±9 % to 114 %±7 % and the EC50 was increased from 10 nmol/L up to 30 nmol/L, P<0.05.

As shown in Fig 5, the NPY induced concentration-response curves were constructed on the top of angiotensin II induced contraction, in which the angiotensin II 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 response by NPY from 92 %±4 % to 78 %±4 %, 68 %±5 % and 41 %±4 %, respectively. Obviously the inhibition caused by tetrangrine was non-competitive.

Effects of tetrangrine on responsiveness of guinea-pig trachea Leukotriene C4, D4 and E4 caused concentration dependent contraction of guinea pig tracheal segments. LTC4- and LTD4-induced concentration-response curves were not significantly influenced by TET (Fig 6). However, the concentration-effect curve produced by LTE4 was significantly shifted rightward by tetrangrine. The calculated dissociation constant (pKb) for tetrangrine against LTE4 receptors was 5.29±0.11.

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

Fig 4. Effect of tetrangrine on angiotensin II-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.

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

DISCUSSION

That tetrangrine as a vasodilator and antihypertensive in the Chinese armamentarium for cardiovascular diseases 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. Not until recently it was revealed that tetrangrine combined with K+ (in a
still unclear relationship, but probably permissively) to promote a neuronal and \( \alpha_1 \)-adrenoceptor mediated contraction [1].

Changes in intercellular \( \text{Ca}^{2+} \) concentration \([\text{Ca}^{2+}]_i\) are critical in smooth muscle contraction or relaxation and the initiation of other cellular responses. The regulation of \([\text{Ca}^{2+}]_i\) involves a complex interaction between \( \text{Ca}^{2+} \) entry and extrusion across the plasmalemma and \( \text{Ca}^{2+} \) release and re-uptake from the sarcoplasmic reticulum. This \( \text{Ca}^{2+} \) movement is controlled mainly by \( \text{Ca}^{2+} \) channels. There are several types of voltage-dependent \( \text{Ca}^{2+} \) channels, including L-type, T-type, N-type and P-type, which differ in structure, function, and pharmacological properties [15]. Voltage-dependent \( \text{Ca}^{2+} \) channels on smooth muscle cells are usually of the L-type, whereas the release of intracellular \( \text{Ca}^{2+} \) is controlled by IP\(_3\) receptors on the sarcoplasmic reticulum [16]. It had been suggested that hypoxia may close oxygen-sensitive K\(^+\) channels which leads to smooth muscle depolarization and \( \text{Ca}^{2+} \) entry, inducing contraction. Functional studies also suggest that \( \text{Ca}^{2+} \) channels are important in the regulation of pulmonary vascular tone. It was found that Bay K8644, a dihydropyridine \( \text{Ca}^{2+} \) agonist, potentiated the constrictor response to angiotensin II or PGF\(_{2\alpha}\). However, the constriction effect on hypoxic pulmonary hypertension is greater than on angiotensin II contraction, suggesting that hypoxic pulmonary vasoconstriction is more dependent on extracellular \( \text{Ca}^{2+} \) [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. 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.

A recent study examined the effects of bosentan, an orally active antagonist of endothelin-A and -B, on the development and maintenance by hypoxia (10 % O<sub>2</sub>)-induced pulmonary hypertension and vascular remodelling in the rat. Pre-treatment with bosentan (100 mg·kg<sup>-1</sup>·d<sup>-1</sup>, 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<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> have 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. 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<sub>4</sub> in newborn lambs and goats mediates its pressor effects via TxA<sub>2</sub> release but has no role in hypoxic pulmonary hypertension. In contrast, in the rat, LTC<sub>4</sub> and LTE<sub>4</sub> have been shown to cause sustained pulmonary pressor responses independent of cyclooxygenase products, albumin or blood. 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 rat<sup>3</sup>. The present study demonstrated that tetrandrine produced a competitive antagonism on leukotriene E<sub>4</sub> rather than leukotriene C<sub>4</sub> and leukotriene D<sub>4</sub> induced contractile responses of guinea pig tracheal segment. The antagonistic activity of tetrandrine on leukotriene E<sub>4</sub> 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

