Tetrandrine and related bis-benzylisoquinoline alkaloids from medicinal herbs: cardiovascular effects and mechanisms of action

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ABSTRACT Tetrandrine (TET), a bis-benzylisoquinoline alkaloid purified and identified an active ingredient in a Chinese medicinal herb, Radix Stephanae tetrandrae, has been used traditionally for the treatment of congestive circulatory disorder and inflammatory diseases. TET, together with a few of its structural analogues, has long been demonstrated to have antihypertensive action in clinical as well as animal studies. Presumably, the primary anti-hypertensive action of TET is due to its vasodilatory properties. TET prevents or inhibits vascular contraction induced by membrane depolarization with KCl or α-adrenoceptor activation with phenylephrine (PE). TET (30 µmol/L) also inhibits the release of endothelium-derived nitric oxide (NO) as well as NO production by inducible NO synthase. TET apparently inhibits multiple Ca²⁺ entry pathways as demonstrated in cell types lacking the L-type Ca²⁺ channels. In cardiac muscle cells, TET inhibits both L- and T-type Ca²⁺ channels. In addition to its actions on cardiovascular tissues, TET may also exert its anti-hypertensive action via a Ca²⁺-dependent manner on other tissues intimately involved in the modulation of blood pressure control, such as adrenal glands. In adrenal glomerulosa cells, KCl- or angiotensin II-induced aldosterone synthesis is highly dependent on extracellular Ca²⁺. Steroidogenesis and Ca²⁺-influx in bovine adrenal glomerulosa cells have been shown to be potently inhibited by TET. In bovine adrenal chromaffin cells, TET inhibits Ca²⁺ currents via L- and N-type channels as well as other unidentified channels with IC₅₀ of 10 µmol/L. Other than the Ca²⁺ antagonistic effects, TET also interacts with the α-adrenergic receptors and muscarinic receptors based on functional as well as radioligand binding studies. Apart from its functional effects, TET and related compounds also exert effects on tissue structures, such as remodelling of hypertrophied heart and inhibition of angiogenesis, probably by causing apoptotic responses. TET is also known for its anti-inflammatory and anti-fibrogenic actions, which make TET and related compound potentially useful in the treatment of lung silicosis, liver cirrhosis, and rheumatoid arthritis.

INTRODUCTION

Cell Ca²⁺ as a target in the treatment of human diseases It is beyond any doubt that cell calcium, in its ionic form Ca²⁺, plays a pivotal role in the mainte-
nance of the normal physiology of living cells and tissues. Disturbance of cellular Ca\(^{2+}\) homeostasis can lead to dysfunction, injury and cell death. Since Ca\(^{2+}\) is an ubiquitous cellular as well as intracellular messenger and altered handling of Ca\(^{2+}\) is often associated with a number of pathophysiological conditions in diseases including cardiovascular diseases\(^{[1,2]}\). Ca\(^{2+}\) signalling pathway has become an important therapeutic target for medicinal drugs. Typical example is the discovery and development of Ca\(^{2+}\)-channel blockers (or Ca\(^{2+}\) antagonist) as effective drugs for the treatment of arrhythmia, hypertension and neurodegenerative diseases. This has also stimulated considerable interest in the study of medicinal herbs and other natural resources traditionally known to possess therapeutic effects on various diseases. Furthermore, understanding of the pharmacological profiles of the active chemical ingredients extracted and/or purified from the traditional medicine may provide an alternative approach towards new drug development.

**More old herbal drugs are found to act on cellular Ca\(^{2+}\)** During the past two decades, the successful development of synthetic dihydropyridine Ca\(^{2+}\) antagonists as novel drugs in effective treatment of various cardiovascular diseases has prompted active search of naturally occurring Ca\(^{2+}\)-antagonists. Not all the previous claims about the Ca\(^{2+}\)-antagonistic effects of these traditional medicine-derived drugs have been verified or confirmed, and very few of these natural product drugs possess better therapeutic or economical advantage over the existing synthetic drugs. Nevertheless, interesting findings and novel observations have indeed emerged from many of such studies on the active ingredients extracted from these traditional medicines. For one example, tetramethylpyrazine (TMP), which is an amide alkaloid of a medicinal plant in China (Ligusticum wolkichii Franchat) and in Africa (Jatropha podagrica), and also present as a natural product from the culture of a strain of Bacillus subtilis\(^{[3]}\), was known to elicit hypotensive action\(^{[4]}\), probably via its inhibitory effects on vasoconstriction\(^{[5,8]}\) and platelet aggregation\(^{[9]}\). TMP also causes negative chronotropic and inotropic responses on isolated atria\(^{[10]}\). Due to its vasodilatory actions particularly in small arterioles, TMP (also known as Ligustazine) has been used in form of infusion solution to treat occlusive cerebral arteriolar diseases\(^{[11]}\). It was suggested that TMP might exert its vasodilatory effect as an α-adrenoceptor antagonist\(^{[12]}\) or as a Ca\(^{2+}\) antagonist blocking L-type Ca\(^{2+}\) channels\(^{[11,12]}\).

However, this claim was not very well supported by our studies. Using several TMP analogues, we provided evidence that TMP did not act as a typical antagonist of the L-type Ca\(^{2+}\) channel and may exert multiple effects on ion channels, including K\(^{-}\)-channels\(^{[18]}\). Furthermore, TMP also acts competitively at the level of α\(_a\)-adrenoceptor\(^{[11,13]}\) and modulates plasma membrane function by affecting the membrane fluidity\(^{[9,14]}\).

The vasodilatory effect of the total glycosides (also referred to as ginsenosides or ginseng saponins) extracted from the dried roots of Panax notoginseng represents another interesting example in that these ginsenosides selectively inhibited vasoconstriction and Ca\(^{2+}\) uptake induced by agonist like phenylephrine, without any inhibitory effect on either the contraction or Ca\(^{2+}\) uptake by KCl-depolarized vascular smooth muscle\(^{[15,16]}\). These results suggest that ginsenosides may be acting selectively on the putative receptor-operated Ca\(^{2+}\) channels. These ginsenosides have also been shown to lower blood pressure in experimental animals presumably due to their vasodilatory effects\(^{[17]}\).

Of the many Ca\(^{2+}\)-acting drugs derived from the traditional medicinal herbs possessing antihypertensive and vasodilatory effects, tetrandrine (TET) and related bisbenzylisoquinoline alkaloids represent a class of old drugs with intensive research in both East and West. Discovery of new pharmacological actions is still evolving from TET and forms the basis for the rest of the discussions.

**Bis-benzylisoquinoline alkaloids in Chinese medicinal herbs** TET is a purified vasodilatory bis-benzylisoquinoline alkaloid derived from the roots of Stephania tetrandra S Moore, a medicinal plant widely distributed in China. Other medicinal plants also contain such structurally similar plant alkaloids with varying biological activities. Chemical structures of several bis-benzylisoquinoline alkaloids are shown in Fig 1. Some more derivatives have been synthesized and they all show antihypertensive actions\(^{[18]}\), but they are not yet commercially available.

**Tetrandrine as an antihypertensive drug** TET was shown more than 3 decades ago, for the first time, to lower systolic as well as diastolic blood pressures in normal subjects without effecting heart rate\(^{[19]}\). These findings were later confirmed in a larger population including hypertensive patients\(^{[20]}\). Similar observations have also been made recently in animals with experimental\(^{[21]}\) and genetic\(^{[18]}\) hypertension. Since the pharmacological actions of TET were described pri-
Fig 1. The chemical structures of a number of bis-benzylisoquinoline alkaloids isolated from Chinese medicinal herbs. These alkaloids contain one to three esteric bond linkages between the two isoquinoline nuclei, offering different degrees of rotational freedom around the ester bond. Thus, some of these alkaloids tend to adopt two or more thermodynamically stable conformational states. (Continued)
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marily in cardiovascular tissues (eg, cardiac and smooth muscles) with special reference to its Ca\(^{2+}\) antagonism at the slow L-type Ca\(^{2+}\) channel sites\(^{22}\), the antihypertensive action of TET has been attributed to the blockade of L-type Ca\(^{2+}\) channels, as earlier shown with verapamil as well. Qian et al\(^{21}\) have made an interesting observation in that, in pithed rats, TET injected intra-arterially dose-dependently impaired the increase in diastolic pressure caused by iv B-HT 920, a highly selective \(\alpha_2\)-adrenoceptor agonist. In low dose, a parallel displacement to the right was observed, whereas for higher dose the shift was non-parallel. These findings suggest that the hypotensive and antihypertensive effects of TET may involve Ca\(^{2+}\) channels as well as \(\alpha_2\)-adrenoceptors.

**Tetrandrine as a Ca\(^{2+}\) antagonist**  
Ca\(^{2+}\)
antagonists, which inhibit the influx of Ca\textsuperscript{2+} into many cell types thereby prevent Ca\textsuperscript{2+} overload-induced cell injury, have been widely used in the treatment of cardiovascular diseases, especially in hypertension. The ability of TET to decrease the Ca\textsuperscript{2+}-dependent component of the cardiac action potential\cite{23} suggests that TET directly interacts with the cardiac L-type Ca\textsuperscript{2+} channels. This is strongly supported by radio ligand binding studies using cardiac muscle plasmalemmal membranes\cite{23}. In uterus and vascular smooth muscle\cite{22,24-27}, TET was found to elicit similar inhibitory effects as, but much less potent than, verapamil (also a synthetic Ca\textsuperscript{2+} antagonist) on KCl-induced contraction (which acts by opening the voltage-dependent L-type Ca\textsuperscript{2+} channels on the smooth muscle cell membranes). However, increasing evidence has suggested that TET should no longer be considered as a selective antagonist for the L-type Ca\textsuperscript{2+} channels as previously claimed. In fact, recent electrophysiological and optical studies with the use of many voltage-dependent channel-selective toxins strongly indicate that TET also inhibits T-type\cite{28,29}, N-type\cite{30} and other poorly defined\cite{31} voltage-dependent Ca\textsuperscript{2+} channels in a variety of tissues with IC\textsubscript{50} values within the concentration range of 4-20 \mu mol/L.

It is interesting to note that the inhibition by TET of Ca\textsuperscript{2+} entry resulting from depletion of intracellular stores with thapsigargin or cyclopiazonic acid (both are selective SR Ca\textsuperscript{2+}-ATPase pump inhibitors) in non-excitable cells, such as HL-60 and murine B lymphoma M12.4 cells\cite{32} devoid of voltage-dependent Ca\textsuperscript{2+} channels, suggests that TET possess a wide non-selective spectrum of Ca\textsuperscript{2+}-antagonistic actions. Up to this date, the study of non-voltage dependent, putative receptor-operated Ca\textsuperscript{2+} channel is hampered by the lack of effective and selective inhibitors. Tetrandrine may be useful for such studies, especially in cells and tissues that do not contain the voltage-dependent Ca\textsuperscript{2+} channels.

**EFFECTS ON VASCULAR SMOOTH MUSCLE**

**Tetrandrine acts on multiple Ca\textsuperscript{2+} pathways** It has been well documented in a large variety of blood vessel preparations that TET effectively inhibited the contraction in KCl-depolarized vascular strips, in a paralleled fashion as demonstrated with verapamil\cite{24,25}. We have studied the effects of TET on the rat aortic muscle contraction and compared to those of nifedipine\cite{26,27}, also a potent inhibitor of L-type Ca\textsuperscript{2+} channels like verapamil except that nifedipine works at the dihydropyridine subdomain of the channel molecule. We found that TET behaved qualitatively similar to, but was less potent than, nifedipine in that it inhibited KCl-induced contraction in a concentration-dependent fashion and its inhibitory effect was long-lasting. However, the effects on phenylephrine-induced contraction of TET was different from those of nifedipine in that the extracellular Ca\textsuperscript{2+}-dependent contraction was inhibited by TET, but not by nifedipine. TET (60 \mu mol/L) completely inhibited the \textsuperscript{45}Ca\textsuperscript{2+} uptake induced by KCl and PE in rat aortic strips\cite{27}. When the aortic muscle contraction was induced by the addition of Ca\textsuperscript{2+} following depletion of intracellular stores by PE in the presence of Ca\textsuperscript{2+}-free medium, TET (60 \mu mol/L) was more effective than 1 \mu mol/L nifedipine in inhibiting such a response to extracellularly added Ca\textsuperscript{2+}. Therefore, TET elicited multiple inhibitory actions on the plasmalemmal Ca\textsuperscript{2+} channels. Furthermore, TET, but not nifedipine, also significantly inhibited the contraction to PE in Ca\textsuperscript{2+}-free medium\cite{27}. Such an action may be due to the effect of TET on the handling of SR Ca\textsuperscript{2+} or on the plasmalemmal \alpha\textsubscript{1}-adrenoceptors. However, TET, at higher concentrations (>30 \mu mol/L) has been shown to potentionate vascular constriction induced by caffeine, which are known to cause Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+}-release from the SR\cite{33}. More recently, the mechanism of action of TET for such a potentiation has been reported to involve the inhibition of SR, as the selective SR Ca\textsuperscript{2+} pump inhibitor, cyclopiazonic acid, also mimic the potentiating effect of TET on caffeine-induced contraction of vascular smooth muscle\cite{33}. It cannot be ruled out that TET inhibits PE-induced contraction in Ca\textsuperscript{2+}-free medium by either directly inhibiting the IP\textsubscript{3}-induced Ca\textsuperscript{2+}-release channels or indirectly inhibiting the \alpha\textsubscript{1}-adrenoceptors.

**Tetrandrine interacts with \alpha\textsubscript{1}-adrenoceptors** During our pharmacological characterization of the vasodilatory effects of TET on endothelium-denuded rings of dog saphenous vein, we have unexpectedly observed a slowly developing transient contractile response upon washout of KCl following the relaxation by TET of KCl-induced contraction\cite{34}. Such a washout-induced contraction in the presence of TET was most prominent in dog saphenous vein, smaller in dog mesenteric vein and not observed in dog mesenteric artery, dog aorta and rat aorta. This transient contraction induced in the presence of TET was not affected by atropine, indomethacin and prazosin, but was sub-
stastically inhibited by phentolamine and rauwolscine. Addition of amiloride or readmission of KCl inhibited the transient contraction upon washout of high KCl in the presence of TET, whereas addition of ouabain turned this transient contraction into a sustained one. It is also interesting to note that the magnitude of this rauwolscine-sensitive, TET-induced transient contractile response in dog saphenous vein, mesenteric vein, mesenteric artery and aorta was closely associated with the number of binding sites (in a descending order) for [3H]-rauwolscine in membranes prepared from these blood vessels. A similar TET-induced contraction has also been observed recently in perfused rat tail artery, but it was sensitive to prazosin, not rauwolscine. Collectively, our results suggest that TET, also elicited a novel contractile effect, which is associated with the activation of postjunctional α-adrenoceptors in some selective blood vessels, but its contractile response is masked by its vasodilatory effect via the blockade of Ca2+ channels, thus appearing transient. It is likely that such small stimulating effect of TET on α-adrenoceptors is unlikely to be of special pharmacological significance, especially in view of its more potent inhibitory action on the same α-adrenoceptors on other blood vessels (see below).

Results from the above in vitro contractility studies using dog vascular tissues and the in vivo presser studies using pithed rats clearly indicate that TET also interacts with α1- and α2-adrenoceptors. However, using radioligand binding of [3H]-prazosin (an α1-adrenoceptor antagonist) and [3H]-clonidine (an α2-adrenoceptor agonist) to rat cerebral membranes, Qian et al failed to observe any effect of TET on [3H]-clonidine binding and found that TET displaced [3H]-prazosin binding with an IC50 value of about 3 μmol/L, comparable to or slightly lower than that for the various Ca2+ channels (4-10 μmol/L). These observations were inconsistent with their in vivo studies of the pressor effects of TET. This may be due to the problems associated with different ligands ([3H]-clonidine vs B-HT 920) and tissues (vascular system vs brain tissue) used in these different experiments. We have investigated the effects of TET on [3H]-prazosin and [3H]-rauwolscine (a selective, high affinity α2-antagonist) binding to plasmalemmal enriched microsomes isolated from dog aorta (high in [3H]-prazosin binding; see Ref 34) and dog saphenous vein (high in [3H]-rauwolscine binding; see Ref 35). Our results (unpublished) show that 10 μmol/L TET was effective in blocking both the binding of [3H]-prazosin and [3H]-rauwolscine to the isolated vascular muscle microsomes thus demonstrating a direct action of TET on both α-adrenoceptor subtypes in vascular smooth muscle, in agreement with the contractility studies.

EFFECT ON VASCULAR ENDOTHELIUM

Tetrandrine inhibits endothelium-dependent relaxation Vascular endothelium is known to cause vasodilation or inhibition of vasoconstriction by releasing nitric oxide (previously termed endothelium derived relaxing factor) via mechanisms that elevate the cytosolic Ca2+ concentration in endothelial cells. Su has recently demonstrated that a TET analogue, 7-O-ethyl-tetrandrine (7-O-TET), was capable of inhibiting the contractile responses of rabbit aorta to norepinephrine (NE) in a concentration-dependent manner. The inhibitory effect of 7-O-ethyl TET was much greater in endothelium-denuded strips than in endothelium-intact strips. Also, 30 μmol/L 7-O-ethyl TET completely blocked the vascular relaxation induced by 0.1 μmol/L ACh, which was added at the plateau phase of the contraction elicited by 0.03 μmol/L norepinephrine. The author attributed the inhibitory effect of 7-O-ethyl TET on ACh-induced relaxation to the antagonism of muscarinic receptor on endothelial cells. This interpretation receives support from showing that TET modulated the negative chronotropic effects of muscarinic agonists and the radioligand binding study of radiolabelled muscarinic antagonists using rat brains.

We have also investigated the functional effects of TET on the endothelial cells of rat aorta and perfused rat mesenteric arteries by examining its ability to modulate the endothelium-dependent vascular relaxation in response to ACh. Addition of ACh (1-3 μmol/L) nearly completely inhibited the contractile responses induced by 1-3 μmol/L phenylephrine and the inhibition was totally reversed by 300 μmol/L Nω-nitro-L-arginine-methylester (L-NAME), which is a selective inhibitor of nitric oxide synthase (NOS), which is a calcium-dependent enzyme catalysing the conversion of the amino acid L-arginine to L-citrulline and nitric oxide, also known as the endothelium-derived relaxation factor. This suggests that the endothelial cells are primarily intact and the enzymatic generation and the subsequent release of nitric oxide (NO) from the endothelial cells are indeed responsible for the ACh-induced relaxation. Pretreatment of 10-30 μmol/L TET inhibited the phe-
nylephrine-induced contraction (by about 50 %–75 %) in both vascular preparations, but the subsequent relaxant effects of ACh was little affected, especially in the rat mesenteric artery preparation. Discrepancies between the above studies were not clear, since vascular tissues from different animal species and different bis-benzylisoquinoline derivatives were employed. If TET indeed inhibits the vascular relaxation via reduction of nitric oxide release from the endothelial cells and at the same time provokes vascular relaxation via direct action on the vascular smooth muscle, the result of these studies based on the contractile responses can be anticipated to be variable depending upon the relative potency of TET on endothelium and vascular smooth muscle cells. One must also consider the involvement of other endothelial-derived relaxation factor which may not be sensitive to the action of tetrandrine. This aspect deserves more detailed studies using several TET analogues, which may differentially affect Ca\(^{2+}\) channels in vascular smooth muscle and endothelial cells.

In the above study, when we used ACh to fully relaxed the endothelium-intact vascular preparations precontracted with 3 µmol/L phenylephrine and challenged the preparations with 10-60 µmol/L TET, TET caused a rapid transient contraction followed by subsequent return to the relaxed state (note that in endothelium-denuded preparations, TET caused only vasorelaxation of phenylephrine-contraction). The biphasic effect of TET in ACh-relaxed preparations could also be mimicked by sequential applications of atropine/TET or L-NAME/TET, but atropine or L-NAME alone caused only vasoconstriction. This TET-induced transient vasoconstriction was also observed in preparations relaxed with ATP (acting on purinoreceptors causing elevation of cytosolic Ca\(^{2+}\) leading to activation of NOS), histamine (acting on histaminergic receptors and subsequent elevation of cell Ca\(^{2+}\) leading to activation of NOS) or thapsigargin (TSG; a potent inhibitor of Ca\(^{2+}\) sequestration thus elevating cytosolic Ca\(^{2+}\) level leading to activation of NOS and the release of NO), but not those relaxed with the Ca\(^{2+}\) ionophore A23187 (which elevates cellular Ca\(^{2+}\) via non-receptor, non-enzymatic route), sodium nitroprusside (generating exogenous NO) or nifedipine (inhibits smooth muscle by inhibiting voltage-dependent Ca\(^{2+}\) channels, which is not present in the endothelial cells). In Fura-2 loaded cultured vascular endothelial cells, TSG induced rapid elevation of cytosolic Ca\(^{2+}\) concentration, and this elevation of cytosolic Ca\(^{2+}\) was inhibited by tetrandrine or its analogue hernandezine\(^{[41,42]}\).

Tetrandrine has also been shown to block Ca\(^{2+}\)-activated K\(^{+}\)-channels\(^{[43]}\). Using an inside-out patch clamp model, Wang \etal\(^{[44]}\) showed that TET elicited a concentration-dependent dual effect on Ca\(^{2+}\)-activated K\(^{+}\)-channels of pulmonary artery smooth muscle cells, decreasing the opening possibility and opening time at higher concentration while at lower concentrations it increased the opening possibility and decreased the close time. Using human endothelial cell lines, Wu \etal\(^{[45]}\) showed that TET suppressed a component of the K\(^{+}\) outward current, believed to be Ca\(^{2+}\)-activated K\(^{+}\)-current and they believed that such an inhibition of K\(^{+}\) currents by TET may contribute to the effect of TET on the functional activities of endothelial cells. Furthermore, in whole-cell configuration of the patch-clamp study using the bovine pulmonary artery endothelial cells, TSG caused a significant enhancement of depolarization evoked Ca\(^{2+}\)-dependent, outward K\(^{+}\) currents, which could also be abolished by TET. These results directly demonstrated that TET, in addition to its known inhibitory effects on vascular smooth muscle by virtue of its Ca\(^{2+}\) antagonistic actions, also inhibits NO production by the endothelial cells possibly by blockade of Ca\(^{2+}\) release-activated Ca\(^{2+}\) channels\(^{[42]}\). Although the effects of TET on vascular endothelial cells enable a more in-depth understanding of the mechanisms of actions of TET at the cellular and membrane level, the inhibition of NO in vascular endothelial cells leading to transient vasoconstriction is overwhelmed by its sustained direct action of vasorelaxation on the vascular smooth muscle cells and may not have much physiological significance.

**Tetrandrine inhibits angiogenesis** An interesting, and potentially important, finding concerning the effect of TET on endothelial cells is the inhibitory action of TET on angiogenesis, the formation of capillaries from the existing blood vessels\(^{[46,47]}\). The inhibitory effect of tetrandrine on tube formation was found to be more than 100-fold greater than that of hydrocortisone\(^{[46]}\). Tetrandrine (10-30 mmol/L) inhibited the tube formation stimulated by interleukin (IL)-1α and platelet-derived growth factor (PDGF)-BB to a greater extent than fetal bovine serum (FBS)-stimulated tube formation. The inhibitory effects of TET on the action of IL-1α and PDGF-BB were non-competitive. These results demonstrate that tetrandrine may reduce the tube formation of endothelial cells in the angiogenic process through inhibition on the post-receptor path-
way of IL-1α and PDGF-BB in chronic inflammation and provide a sound basis for its possible use in the control of vascularization in tumor growth or diabetic ocular diseases. The anti-angiogenic effect of TET may be related to its ability to inhibit the non-voltage dependent Ca²⁺-entry pathway. For example, carboxamidotriazole (CAI), an inhibitor of non-voltage dependent Ca²⁺ channels like TET, was also found to inhibit the proliferation of human umbilical vein endothelial cells in response to serum or basic fibroblast growth factor [58].

**EFFECTS ON ADRENAL GLANDS**

**Inhibition of steroidogenesis and Ca²⁺ influx in glomerulosa cells** In outer zona glomerulosa cells of the adrenal cortex, angiotensin II (AII) or KCl depolarizes the cell [49,50] and induces aldosterone synthesis, which is highly dependent on the elevation of cytosolic Ca²⁺ concentration [51, 52] as a result of entry of Ca²⁺ [53]. Therefore, voltage-dependent Ca²⁺ channels appear to be involved in the regulation of steroidogenesis and partly contribute to its antihypertensive effect [49,54]. Recently, it has been demonstrated that TET inhibited aldosterone production induced in bovine adrenal glomerulosa cells by low concentration of KCl (15 mmol/L; not sufficiently concentrated to activate the L-type Ca²⁺ channels) and AII (100 nmol/L) in a concentration-dependent manner with IC₅₀ value of about 10 µmol/L [29].

In addition, in studies using Fura-2 loaded bovine adrenal glomerulosa cells, TET inhibited the tonic Ca²⁺ influx phase induced by KCl and AII without effecting the initial transient Ca²⁺ release phase. Using the whole cell configuration of the patch clamp technique, the effect of TET on voltage-activated Ca²⁺ currents was investigated under conditions when K⁺ and Na⁺ currents were absent. T-type currents were isolated from the L-type currents by measuring the slowly deactivating tail currents developed upon repolarizing the cell to -65 mV following various depolarizing pulses [54]. These tail currents were inhibited by TET in a concentration-dependent manner (IC₅₀=9 µmol/L), but the voltage sensitivity of channel activation was not affected by TET. Higher concentrations of TET also inhibited the L-type currents. Since TET blocked T-type channels and inhibited aldosterone production with a very similar potency, these two effects may be causally related. TET has a unique structure with preference for T-type Ca²⁺ channels, whereas dihydropyridine Ca²⁺ antagonists have a clear selectivity for L-type Ca²⁺ channels. Since L-type Ca²⁺ channel is present in great abundance in vascular smooth muscle, this could explain why excitation-contraction coupling in vascular smooth muscle is more sensitive to dihydropyridine than stimulation-secretion coupling in adrenal glomerulosa cells [55]. It is conceivable that the therapeutic antihypertensive effects may be partly due to its inhibition on aldosterone production.

**Inhibition of multiple Ca²⁺ currents in chromaffin cells** The chromaffin cells in the adrenal medullary are known to be involved in the release of catecholamines, which also depends on extracellular Ca²⁺ [56]. Dysfunction of catecholamine release by chromaffin cells as in pheochromocytoma can also lead to severe hypertension. The widely studied bovine chromaffin cells elicit no low-voltage activated, but several types of high-voltage activated Ca²⁺ channel currents as demonstrated by recent studies using radioligand binding and electrophysiological techniques [56-59]. These Ca²⁺ channels include α-conotoxin-sensitive N-type Ca²⁺ channels [60], dihydropyridine-sensitive L-type Ca²⁺ channels [58] and FTX-sensitive P-type Ca²⁺ channels [58]. TET blocked the Ca²⁺ channel currents almost completely with IC₅₀ occurring at about 10 µmol/L [31]. The current-voltage relationship was not shifted by TET and transient Ca²⁺ currents with a prominent initial rise were observed during the tetrandrine block, suggesting that TET may block the open Ca²⁺ channels and dissociate slowly. Unlike in adrenal glomerulosa cells, the current block is use-dependent and takes longer time, 8-10 min, to achieve its full inhibitory effects. These authors suggested that TET, being lipophilic, possibly acts from intracellularly rather than extracellularly [31]. This possibility may be better explored with membrane patches of different orientations. It is also possible that TET affects low-voltage activated and high-voltage activated Ca²⁺ currents differently. The effect of TET on the release of catecholamines by the chromaffin cells has recently been found to be reduced by TET due to the inhibition of the voltage-operated Ca²⁺ channels [60]. However, in the same study, the cytosolic Ca²⁺ concentration was found to be increased by TET. The authors attributed this observation to the inhibition by TET of endoplasmic reticulum Ca²⁺ pump activity.

**OTHEREFFECTS OF TETRANDRINE**

**Tetrandrine and glutamate receptors** The role of the glutamate receptor in mediating neuronal damage
following cerebral ischaemia, is now well established and the cell damage/death may involve dysfunction of mitochondria and Ca$^{2+}$ regulation$^{[61]}$. Glutamate promotes excitotoxicity in a process that involves the production of large quantities of NO through the mediation of inducible NOS$^{[62,63]}$. Several authors have reported that tetrandrine has the capacity to minimize or protect the brain from ischaemic/anoxic insult. With the aid of the patch clamp technique, Wang et al$^{[65]}$ showed that rat cortical neurons were protected from anoxic damage through the inhibition of the opening of NMDA receptor channels by tetrandrine. Che et al$^{[64]}$ showed that tetrandrine protected fetal rat cortical neurons from injury induced by excitotoxins, such as glutamate, beta- N-oxalylamino-L-alanine (BOAA, on non-NMDA receptors) and beta-N-methylamino-L-alanine (BMMA, on NMDA receptors). However, they could not demonstrate tetrandrine protection with N-methyl-D-aspartate (NMDA) excitotoxicity in their model. They suggested that the protective effect of tetrandrine was due to its ability to inhibit the cytosolic free Ca$^{2+}$ overload that follows neuronal insult. Similar results were obtained by Xuan et al who showed that tetrandrine inhibited the increase in cytosolic Ca$^{2+}$ that was induced by glutamate in rabbit retina cells$^{[65]}$. Glutamate excitotoxicity is a major pathophysiologic process of stroke and its inhibition by tetrandrine means that this drug has a good potential to be useful in the management of stroke. As with glutamate excitotoxicity, apoptosis also involves increases in cytosolic Ca$^{2+}$, the inhibition of which is partly the basis for the therapeutic use of tetrandrine in pathologic conditions involving apoptosis.

**Apoptotic, antioxidant and anti-inflammatory effects of tetrandrine** Apoptosis is a natural mechanism for the regulation of tissue growth. It is the process by which cells die under genetic control, hence it is also called programmed cell death or cell suicide. It regulates tissue growth ensuring that tissues do not grow out of control (causing for example, cancer). It arrests tissues at certain levels of growth, thereby promoting tissue differentiation, and some autoimmune diseases are known to be associated with defects in apoptosis$^{[66]}$. The process of apoptosis is reported to be part modulated by oxygen free radicals, which are known to contribute to the oxidative stress syndrome. A drug that modifies apoptosis, therefore, has the potential to influence tissue growth and, therefore the treatment of cancer and/or the modification of the aging process which is partly attributable to oxidative stress$^{[67]}$. It can also serve as an antioxidant and anti-inflammatory agent by modifying the effect of oxygen free radicals. The use of tetrandrine and its analogues in the treatment of autoimmune disease (such as arthritis) has recently been explained on the basis of their ability to induce apoptosis and to suppress peripheral blood T-cells which are known to mediate the autoimmune response$^{[68]}$. Tetrandrine has a dual cytotoxic and cytoprotective effect based on the dose. Its cytotoxic effect is reported to be due to reactive oxygen species (H$\text{}_2\text{O}_2$)-induced apoptosis while its cytoprotective effect could partly be due to inhibition of intracellular calcium influx$^{[69]}$. Its apoptotic effect has been demonstrated as a potential basis for chemotherapy of hepatocellular carcinoma$^{[70]}$. However, Dong et al$^{[71]}$ reported that TET elicited concentration-dependent inhibition of growth of both normal human peripheral blood lymphocytes and human leukemia HL-60 cells. Morphological observation and DNA analysis revealed that TET and its structural analogue, berbamine, caused cell shrinkage with the formation of apoptotic bodies, and showed clear evidence of DNA fragmentation, indicating the induction of cell death via apoptosis. TET has also been reported to be useful in the treatment of retinal degeneration resulting from ocular ischaemia and inflammation. The mechanism of this effect is through the inhibition of interleukin-1, leading to an anti-inflammatory effect that is more potent than is obtained by the corticosteroid, prednisolone$^{[72]}$. The anti-inflammatory effects of TET and related alkaloids have been reviewed in a greater detail in other contributions in this special issue.

**CONCLUSIONS**

In this communication, we have reviewed some of the pharmacological profiles of TET, a structurally unique Ca$^{2+}$ antagonist of Chinese medicinal herb origin with therapeutically antihypertensive action. We have noted several general points in the consideration of development of new therapeutic drugs or pharmacological tools from the natural resources. First, the antihypertensive and vasodilatory actions of TET are consistent with its pharmacological actions at the tissue and membrane levels and are closely associated with its action on cellular Ca$^{2+}$ mobilization. Second, TET elicited a wide spectrum of Ca$^{2+}$ channel antagonism with a preferential action on L-type and T-type Ca$^{2+}$ channels;
in addition, increasing evidence shows that TET also modulates intracellular Ca\(^{2+}\) stores. Third, other pharmacological effects at the membrane receptors, particularly the \(\alpha\)-adrenoceptors, should be taken into consideration in the vasodilatory and anti-hypertensive actions of TET. Fourth, the tissue selectivity (e.g., heart versus arteries, and arteries versus veins) of TET action warrants future investigations. Fifth, as in the case for many natural products, the relatively weak pharmacological potency may post limitation of their clinical use. However, its synergetic action with other ingredients in the same plant (or other herbs) remains an area of potential interest and importance. Finally, although TET is relatively safe within its therapeutic range, it has been undeservingly tagged with the label of “Chinese herb nephropathy” due to the presence of aristolic acid from adulterated herbal products. Finally, despite the relatively broad spectrum and weak potency of cellular actions, the \(\text{bis-benzylisoquinoline}\) alkaloids with various configurations and conformations, including TET, which show newly discovered pharmacological actions, have become increasingly useful tools in the study of Ca\(^{2+}\)-signalling mechanisms. It is possible that chemical modification of these lead compounds may give way to future pharmacologically selective and therapeutically potent drugs of clear clinical significance.

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REFERENCES

20. Gao Y, Chang MY, Mao HY, Chao HY, Chen DH. The clinical observation of tetrandrine in the treatment of 270 cases of hypertensive patients and hypertensive crisis. Chin
36 Achike IF, Kwan CY, Shi AG, Kwan CY, Daniel EE. Characterization of a novel tetrandrine-
35 Shi AG, Kwan CY, Daniel EE. Relation between density
34 Kwan CY, Wang ZL. Tetrandrine - a vasodilator of medicinal
33. Kwan CY. Tetrandrine potentiates caffeine-induced
32 Leung YM, Kwan CY, Loh TT. Dual effects of tetrandrine
31 Weinsberg F, Bickmeyer U, Wiegand H. Effects of tetrandrine
30 Wiegand H, Meis S, Gotzsch U. Inhibition by tetrandrine of
28 Liu QY, Karpinski E, Pang PKT. Tetrandrine inhibits both
27 Kwan CY, Deng HW, Guan YY. Tetrandrine is not a selec-
26. Su JY. Mechanisms of action of 7-O-ethyl tetrandrine in
25 Jia JF, Gao NN, Xia GJ, Luo QF, Fang DC, Jiang MX.
tetrandrine on vascular smooth muscle. J Tradit Chin Med
13 Spat A, Enyedi P, Hajnoczky GM, Hunyady L. Generation
12 Kwan CY, Vallotton MB. Blocking T-type calcium channels with
10 Kwan CY, Ma FM, Hui SCG. Inhibition of endothelium-dependent vascular relaxation by tetrandrine. Life Sci 1999; 64: 2391-400.
3 Spat A, Enyedi P, Hajnoczky GM, Hunyady L. Generation
2 Kwan CY et al Acta Pharmacol Sin 2002 Dec; 23 (12): 1057-1068


60 Bickmeyer U, Weinsberg F, Muller E, Wiegand H. Blockade of voltage-operated calcium channels, increase in spontaneous catecholamine release and elevation of intracellular calcium levels in bovine chromaffin cells by the plant alkaloid tetrandrine. Naunyn-Schmiedeberg’s Arch Pharmacol 1998; 357: 441-5.


