Catharanthine Dilates Small Mesenteric Arteries and Decreases Heart Rate and Cardiac Contractility by Inhibition of Voltage-Operated Calcium Channels on Vascular Smooth Muscle Cells and Cardiomyocytes

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ABSTRACT

Catharanthine is a constituent of anticancer vinca alkaloids. Its cardiovascular effects have not been investigated. This study compares the in vivo hemodynamic as well as in vitro effects of catharanthine on isolated blood vessels, vascular smooth muscle cells (VSMCs), and cardiomyocytes. Intravenous administration of catharanthine (0.5–20 mg/kg) to anesthetized rats induced rapid, dose-dependent decreases in blood pressure (BP), heart rate (HR), left ventricular blood pressure, cardiac contractility (dP/dtmax), and the slope of the end-systolic pressure-volume relationship (ESPVR) curve. Catharanthine evoked concentration-dependent decreases (IC50 > 98%) in endothelium-independent tonic responses of aortic rings to phenylephrine (PE) and KCl (IC50 = 28 μM for PE and IC50 = 34 μM for KCl) and of third-order branches of the small mesenteric artery (MA) (IC50 = 3 μM for PE and IC50 = 6 μM for KCl). Catharanthine also increased the inner vessel wall diameter (IC50 = 10 μM) and reduced intracellular free Ca2+ levels (IC50 = 16 μM) in PE-constricted MAs. Patch-clamp studies demonstrated that catharanthine inhibited voltage-operated L-type Ca2+ channel (VOC) currents in cardiomyocytes and VSMCs (IC50 = 220 μM and IC50 = 8 μM, respectively) of MA. Catharanthine lowered BP, HR, left ventricular systolic blood pressure, and dP/dtmax and ESPVR likely via inhibition of VOCs in both VSMCs and cardiomyocytes. Since smaller vessels such as the third-order branches of MAs are more sensitive to VOCC blockade than conduit vessels (aorta), the primary site of action of catharanthine for lowering mean arterial pressure appears to be the resistance vasculature, whereas blockade of cardiac VOCs may contribute to the reduction in HR and cardiac contractility seen with this agent.

Introduction

Catharanthus roseus, also known as Vinca rosea or Madagascar periwinkle, is a tropical shrub containing a wide spectrum of bioactive constituents such as alkaloids, terpenoids, bioflavonoids and tannins (van Der Heijden et al., 2004; Sertel et al., 2011). The crude extract of the plant, popular in folklore medicine, has been purported to possess hypotensive, cerebral vasodilator, antiatherogenic, anti-inflammatory, wound healing, and diuretic properties (Lans, 2006; Nayak et al., 2007; Ara et al., 2009; Rasineni et al., 2010). However, the mechanisms or the active constituents that contribute to these claims are currently unknown. Several oral formulations of Madagascar periwinkle extracts are present as homeopathic or herbal medication in Western countries (Madagascar Periwinkle Monograph; http://naturaldatabase.therapeuticresearch.com/nd/Search.aspx?cs=&s=ND&pt=100&sh=1&id=637). The major bioactive constituents are the indole ring containing catharanthine and the terpenoid-based vindoline alkaloids. Together, these constitute anticancer agents such as vincristine, vinblastine, vinorelbine, and vinflunine, which belong to the class of vinca alkaloids (van Der Heijden et al., 2004; Sertel et al., 2011). Vincristine (0.8–2 mg/m²) and vinblastine (0.1–20 mg/m²) are administered by the i.v. route in milligram

ABBREVIATIONS: ACh, acetylcholine chloride; ANOVA, analysis of variance; BP, blood pressure; BSA, bovine serum albumin; [Ca2+], cytosolic free calcium; dP/dtmax, cardiac contractility; ESPVR, end-systolic pressure-volume relationship; HR, heart rate; L-Wee, L-tryptophan ethyl ester; LVSBP, left ventricular systolic blood pressure; MA, superior mesenteric artery; PE, phenylephrine; SA, sino-atrial; VOCC, voltage-operated L-type Ca2+ channel; VSMC, vascular smooth muscle cell.
Materials and Methods

These studies were conducted in 13-week-old male Sprague-Dawley rats (200–250 g) obtained from Charles River Laboratories (St. Constant, QC, Canada). The experimental protocols approved by the respective animal care committees at both universities conformed to the Guide for the Care and Use of Laboratory Animals stipulated by the Canadian Council on Animal Care and the National Institutes of Health. The rats (n = 14) anesthetized with isoflurane gas (5% induction and 2% maintenance) were maintained on heating pads at approximately 37°C. A thermometer inserted in the rectum before induction and 2% maintenance) were maintained on heating pads at

Materials. Acetylcholine chloride (ACh), bovine serum albumin (BSA), dithiothreitol, EGTA, fura-2 acetoxymethyl ester, indomethacin, PE hydrochloride, papain, as well as all of the salts used in the preparation of various buffers were of analytical grade obtained from either Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) or Bishop & Firkin (Burlington, ON, Canada). Collagenase type II and elastase were obtained from Worthington Biochemical Inc. (Lakewood, NJ). Triiodothyronine sodium was obtained from Abbott Laboratories Ltd. (Saint-Laurent, QC, Canada). Catharanthine was extracted and purified to 99%. This was confirmed by nuclear magnetic resonance and mass spectrometry characterization at the Plant Biotechnology Institute of the National Research Council of Canada. It was used as hydrochloride salt.

Measurement of BP, HR, Intracardiac Pressure, and Cardiac Contractility. Detailed methodology for the measurements of BP, HR, dP/dt max, and left ventricular blood pressure performed in rats that were anesthetized with isoflurane was as previously described (Jadhav et al., 2012; Papageorgiou et al., 2012). Either catharanthine (0.5–20 mg/kg) or saline (vehicle) was administered (in volumes <0.3 ml/kg) via a catheter inserted in the femoral vein. A pressure transducer attached to a catheter (PE50) inserted in the femoral artery recorded BP and HR (Jadhav et al., 2012). The studies performed at the University of Saskatchewan determined the changes occurring in BP and HR after administration of either saline or increasing doses of catharanthine in the same rat. The total volume of vehicle (saline) or catharanthine administered to each rat was kept to a minimum (<1.2 ml) and no more than six doses were given to each rat over a period of 60 minutes. A dose of catharanthine >20 mg/kg was not attempted since it produced a profound fall in BP and HR that did not return to baseline. On the basis of these findings, further studies conducted at the University of Toronto determined the changes in intracardiac pressures (left ventricular systolic blood pressure [LVSBP]) and cardiac contractility (dP/dt max) by advancing pressure-volume catheter (SPR-838; Millar Instruments, Houston, TX) through a cannula into the right common carotid artery into the left ventricle. From the data gathered, it was feasible to calculate the changes in left ventricular volume and the slope of the end-systolic pressure-volume relationship (ESPVR) curves before and after the administration of catharanthine at 1, 3, and 10 mg/kg (Papageorgiou et al., 2012).

Rat Aortic Rings. The detailed methodology for the measurement of vasodilator responses in endothelium-denuded rat aortic rings was provided earlier (Hopfner et al., 1998; Jadhav et al., 2012). Briefly, isolated rat thoracic aortic rings were set up in organ baths containing 10 ml of Krebs buffer [in mM: 120 NaCl, 4.8 KCl, 1.2 MgCl2, 1.2 CaCl2, 1.2 KH2PO4, 25 NaHCO3, and 11 glucose (pH 7.4 gassed with 95% O2, 5% CO2 at 37°C)] maintained under a resting preload tension of 2 g. Steady-state tension responses were elicited to either PE (1 μM) or a depolarizing solution of KCl (100 mM with equimolar NaCl reduced in the bathing buffer) that elicited a submaximal response (approximately EC50–90 level). Once a steady tonic response was reached, cumulatively increasing concentrations of catharanthine (500 nM–160 μM) were added in such a way that the next concentration was added only after the response to the previous concentration had plateaued. Since the presence or the absence of endothelium did not affect the responses to catharanthine, all studies were performed in endothelium-denuded vessels as previously described. The tension responses were recorded in millinewtons on a chart program (Chart V5.0.1) using a Powerlab/SPS data acquisition system (AD Instruments Pty. Ltd., Sydney, NSW, Australia).

Third-Order Branches of Superior MA. The vessels were isolated (i.d., <200 μm) and suspended between a micropositioner and force transducer with stainless steel wires (40 μm diameter) in a myograph chamber, model 610M Multi Wire Myograph System (Danish Myotechnology, Aarhus, Denmark). Resting tension (2 mN) was fixed and the rings were maintained for initial equilibration period of 1 hour in Krebs buffer having the same composition and conditions of incubation as described for aortic rings. The changes in force developed were recorded as the increase in millinewtons on a Powerlab data acquisition system. Endothelium was removed by scratching the intimal layer by passing a human hair a few times through the isolated small MA before setting it up in a wire myograph apparatus. It was considered as denuded if the dilator response to ACh was >10% in PE-constricted MA (10 μM, approximately EC50–90 concentration) (Mishra et al., 2008; Jadhav et al., 2012). The concentration-inhibitory response relationships for cumulative additions of
catharanthine (50 nM–40 μM) were determined under steady-state tonic responses elicited by either PE (10 μM) or KCl (100 mM).

The steady-state tension responses elicited by a depolarizing buffer containing high KCl (100 mM) was prepared with appropriate reduction in NaCl concentration in the buffer to a level of 24 mM with all other salts being present at the same level as indicated above for the normal Krebs buffer. We also determined the responses to depolarizing KCl either in the presence or absence of phentolamine (10 μM) to rule out a role for depolarization-induced norepinephrine release from the sympathetic nerve endings contributing to the altered tension response. It was ascertained in preliminary experiments that the presence or absence of phentolamine did not affect the steady-state tension response to KCl (100 mM). Moreover, the steady tension responses elicited by either PE or high KCl or the vasodilator steady-state tension response to KCl (100 mM). The pipettes had a resistance of 2.0–2.5 MΩ when filled with a solution containing the following (in mM): 115 NaCl, 5.4 CaCl2, 1.0 MgCl2, 5.0 BaCl2, 10 HEPES, and 10 glucose (pH 7.4 adjusted with NaOH). The pipettes had a resistance of 2.0–2.5 MΩ when filled with a solution containing the following (in mM): 115 NaCl, 5.4 CaCl2, 1.0 MgCl2, 5.0 BaCl2, 10 HEPES, and 10 glucose (pH 7.4 adjusted with NaOH). Series resistance was compensated by 80–90%. VSMCs were held at −60 mV and stepped to −40 mV for 300 milliseconds before voltage steps from −60 to +50 mV for 300 milliseconds to examine VOCC currents (Liang et al., 2009; Jadhav et al., 2012). Currents at 0 mV were recorded every 10 seconds during catharanthine perfusion. The mean ± S.E.M. cell capacitance values of VSMCs were 12.9 ± 1.8 pF (n = 8 VSMCs isolated from five rats).

Isolation of Rat Ventricular Myocytes. Single ventricular myocytes were isolated from rat hearts using a protocol we previously described for the mouse (Liang et al., 2010). Hearts were isolated and perfused in a retrograde manner for 5 minutes with nominally Ca2+-free Tyrode solution containing the following (in mM): 136 NaCl, 5.4 KCl, 0.5 Na2HPO4, 10 HEPES, 1 MgCl2, and 10 glucose (pH 7.40 adjusted with NaOH). After perfusion with a Ca2+-free Tyrode solution containing collagenase (1 mg/ml, type II; Worthington) and protease (0.028 mg/ml, type XIV; Sigma-Aldrich) for 7–10 minutes, the left ventricle was dissected, cut into small pieces, and gently triturated to release single myocytes. Isolated myocytes were stored in Krah-Bruhe (KB) solution, containing the following (in mM): 100 potassium glutamate, 10 potassium aspartate, 2.5 KCl, 10 KH2PO4, 2 MgSO4, 5 HEPES, 20 glucose, 20 taurine, 5 creatine, 0.5 EGTA, and 0.1% albumin (pH 7.2, adjusted with NaOH). The cells were used for the study within 8 hours of isolation.

Recording of VOCC currents from Ventricular Myocytes. VOCC currents were recorded from single ventricular myocytes at 22°C using whole-cell patch-clamp technique as described above. The external recording solution contained the following (in mM): 137 NaCl, 5.4 CsCl, 1 MgCl2, 1.2 CaCl2, 10 HEPES, and 10 glucose (pH 7.35 adjusted with NaOH). The pipettes solution contained (in mM): 115 CsCl, 20 TEA-Cl, 1 MgCl2, 10 EGTA, 10 HEPES, 0.2 Na2GTP, and 5 Mg-ATP (pH 7.2 adjusted with CsOH). Myocytes were held at −85 mV and Na+ currents were inactivated by applying a 300-millisecond voltage step to −40 mV before voltage steps from −60 to +50 mV for 400 milliseconds to examine VOCC currents (Liang et al., 2010).

Statistical Analysis. For in vivo study, the percentage (%) maximum fall in BP and HR attained from basal value after administration of each dose of catharanthine was determined in each rat and the pooled values are expressed as mean ± S.E.M. (n = 7 rats). The data indicated as mean ± S.E.M. were analyzed for statistical significance using one-way analysis of variance (ANOVA) as the same variable (BP or HR change) was compared in the same animal before and after the additions of each dose of catharanthine, followed by Tukey’s post hoc test. These results were presented as line graphs. The closest P value obtained is given in the Results. The mean ± S.E.M. data were subjected to statistical significance for differences between means using one-way ANOVA followed by Tukey’s post hoc test when the same variable was compared in the same animal/tissue/cell before and after the administration of catharanthine. The data for comparison of the mean values for the inhibitory effects of catharanthine on PE or KCl-evoked responses were analyzed by two-way ANOVA followed by Tukey’s post hoc test. Statistical comparisons in left ventricular performance parameters performed at the University of Toronto were analyzed using the Sigma Plot program (version 11.0; Systat Software Inc., San Jose, CA). The differences between means was considered significant when P < 0.05.
Results

Intravenous administration of vehicle did not affect the basal BP or HR for a period of 1 hour (Fig. 1). In contrast, administration of catharanthine (0.5–20 mg/kg i.v.) evoked dose-dependent reductions in both BP and HR. The data from a representative tracing are shown in Fig. 2, A and B. At low doses (0.5–5 mg/kg), catharanthine evoked rapid, transient reductions in BP and HR (lasting <2 minutes), whereas at higher doses (10 and 20 mg/kg), the BP and HR reductions were sustained. The maximal reductions in BP and HR were reached after about 10–30 seconds postinjection, respectively. The responses at all concentrations reached stable levels after 5 minutes. The data from several experiments confirmed that BP decreased ($P < 0.01$) by more than 50% from 101 ± 12 mmHg (control value) to 48 ± 7 mm Hg after the administration of the highest dose (20 mg/kg) of catharanthine (Fig. 2C). Similarly, HR decreased ($P < 0.05$) from 399 ± 12 beats/min in control conditions to 297 ± 14 beats/min after the administration of 20 mg/kg catharanthine (Fig. 2D).

It is clear that catharanthine causes sustained dose-dependent reductions in both BP and HR. To further explore the action of catharanthine on the heart, we measured intraventricular pressure-volume relationships using an impedance catheter. The representative data from a single experiment for the fall in LVSBP and the $dP/dt_{max}$ attained after a single dose of catharanthine (10 mg/kg) administration are shown in Fig. 3A. Consistent with the BP results, peak LVSBP were reduced after administration of increasing doses (1, 3, 10 mg/kg dose i.v.) of catharanthine in a concentration-dependent manner by values of 10 ± 1 mm Hg, 17 ± 2 mm Hg, and 23 ± 1 mm Hg at nadir (approximately 30 seconds postinjection), and by 5 ± 1 mm Hg, 8 ± 2 mm Hg, and 9 ± 1 mm Hg at steady state (approximately 5 minutes postinjection), respectively (Fig. 3B). Catharanthine dose-dependently decreased the maximal time derivative of the left ventricular pressure (i.e., $dP/dt_{max}$) with a pronounced transient effect 30 seconds after catharanthine administration, which reached a stable level approximately 5 minutes postinjection (Fig. 3, A and C). In addition to decreasing $dP/dt_{max}$, catharanthine also...

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**Fig. 1.** A representative experiment showing that acute i.v. administration of saline failed to alter BP (A) and HR (B) in a 13-week-old Sprague-Dawley rat for up to 1 hour.

**Fig. 2.** Representative traces showing the acute effects of i.v. administration of increasing doses (0.5–20 mg/kg) of catharanthine on BP (A) and HR (B) in a 13-week-old male Sprague-Dawley rat. Similar patterns of responses were seen in seven rats. The line graphs represent the maximum fall in BP (C) and HR (D) values at varied time intervals after the administration of indicated concentrations of catharanthine (0.5–20 mg/kg i.v.). Each data point is the mean ± S.E.M. ($n = 7$ rats). *$P < 0.05$, **$P < 0.01$ compared with respective basal value before the addition of catharanthine. bpm, beats per minute.
dose-dependently decreased the slope of the ESPVR from 0.18 ± 0.01 mm Hg/µl in the absence of drug to 0.06 ± 0.01 mm Hg/µl (measured approximately 5 min postinjection of 10 mg/kg catharanthine), further demonstrating that catharanthine impairs cardiac contractility (Fig. 4).

Although the effects of catharanthine on heart function (HR and cardiac contractility) could be responsible for the profound reductions in BP, it is conceivable that this agent could also have vascular effects. The addition of Krebs buffer in organ baths failed to affect the steady-state sustained tonic response elicited by a fixed concentration of either PE or KCl in both aortic and small MA rings (Figs. 5 and 6). In contrast, we found that addition of cumulatively increasing concentrations of catharanthine caused progressive reductions in tension of endothelium-denuded preconstricted isolated aortic vessels (Fig. 7) and MAs (Fig. 8). Similar results were observed in both vessels with intact endothelium. It should be noted that the inhibitory effects were slower and generally higher levels of catharanthine were required to inhibit the steady-state tension responses in aortae (IC_{50} = 28 ± 2 µM for PE and IC_{50} = 34 ± 3 µM for KCl) compared with MA (IC_{50} = 3 ± 1 µM for PE and IC_{50} = 6 ± 2 µM for KCl). However, the next concentration of catharanthine was added only after the response plateaued for the previous concentration and as the time scale was crunched to accommodate all of the additions, it is not distinctly clear in the case of aortic rings. However, this is not the case with MA because the inhibitory effect for each concentration was reached quickly and the concentration-response curve showed a clear step-wise ladder pattern. These data revealed that MA was more sensitive (P < 0.01) to catharanthine blockade than aortic rings.

To explore the cellular basis for force inhibition in arterial vessels, we measured the vessel diameter and [Ca^{2+}], level in PE-constricted (10 µM) MA. The data from a single experiment for the decrease in vessel wall diameter (vasoconstriction), along with the increase in fura-2 fluorescence ratio followed by the inhibitory effects on these responses exerted by the addition of increasing concentrations of catharanthine, are shown (Fig. 9, A and B). The concentration-dependent increases in vessel diameter and fura-2 fluorescence ratio after the addition of catharanthine in PE-constricted vessels were calculated from several experiments. These data were compared over the same time course for the responses to PE determined in the absence of catharanthine (Fig. 9, C and D). Catharanthine evoked vasodilatation (IC_{50} = 9.8 ± 1.4 µM) along with parallel decreases in [Ca^{2+}], (IC_{50} = 15.8 ± 2.3 µM) in PE-constricted MA. Interestingly, at lower catharanthine concentrations (i.e., < 5 µM), the oscillatory vasomotor activity typically seen in the vessel diameter and [Ca^{2+}], of MA that have been pretreated with PE are eliminated (Fig. 9, A and B), suggesting that catharanthine could affect vascular tone at lower concentration ranges below those required for global vasodilatation and BP-lowering effects encountered at higher concentrations.

On the basis of the above results, we hypothesized that a common mechanism for the actions of catharanthine on arterial vasculature and heart is the inhibition of VOCC. Consistent with our hypothesis, catharanthine inhibited Ba^{2+} currents (P < 0.01) through VOCCs measured in isolated single VSMCs of the MA (Fig. 10, A and B). Specifically, the Ba^{2+} current density at 0 mv (~3.0 ± 0.3 pA/pF) was reduced (P < 0.01; n = 8 cells) to −1.62 ± 0.3 pA/pF at 3 µM, −0.93 ± 0.2 pA/pF at 10 µM, and −0.26 ± 0.1 pA/pF at 30 µM catharanthine. These data support the conclusion that the BP-lowering and vasodilatory effects of catharanthine occur as a result of inhibition of VOCCs in VSMCs of the MA. Catharanthine also dose-dependently inhibited (P < 0.05) VOCCs in cardiomyocytes (Fig. 11, A and B). Specifically, Ca^{2+} currents through cardiomyocytes at 0 mV were reduced from −4.0 ± 0.5 pA/pF to −2.3 ± 0.5 pA/pF at 100 µM, −1.3 ± 0.3 pA/pF at 300 µM, and −0.45 ± 0.2 pA/pF at 1 mM catharanthine. The inhibitory effect of catharanthine on VOCC currents was less potent (P < 0.01) in cardiomyocytes (IC_{50} = 224 ± 80 µM; Fig. 11C) than in VSMCs from MA (IC_{50} = 8.4 ± 2.5 µM; Fig. 10C).
Discussion

Extracts from *C. roseus* plants are used to treat many conditions, including cancer and diabetes (Lans, 2006; Nayak et al., 2007; Ara et al., 2009; Rasineni et al., 2010; Madagascar Periwinkle Monograph; http://naturaldatabase.therapeuticeareresearch.com/nd/Search.aspx?cs=ND&pt=100&sh=1&id=637). Our studies revealed that catharanthine, a major constituent of *C. roseus* plants, lowers BP, reduces HR, and impairs cardiac contractility in normotensive rats. The hemodynamic and cardiac effects of catharanthine injection were biphasic, characterized by a potent rapid response (<30 seconds) followed by sustained effects at its higher concentrations.

Although the reductions in BP could be partially related to the cardiac actions of catharanthine, we found that catharanthine induces endothelium-independent vasodilatation of MA (IC50 = 3–6 μM) and aortic rings (IC50 = 28–34 μM) with different degrees of sensitivity. The relaxation effects of catharanthine correlated with the inhibition of VOCCs (IC50 = 8.4 μM) in freshly dispersed VSMCs isolated from MAs as well as the reductions in \([\text{Ca}^{2+}]_i\) (IC50 = 15.8 μM) in MA rings. Taken together, these findings are consistent with the conclusion that the dose-dependent drop in BP evoked by catharanthine is caused, at least in part, by endothelium-independent inhibition of smooth muscle contraction in small blood vessels such as MAs as a result of reductions in \([\text{Ca}^{2+}]_i\) arising from the blockade of VOCCs.

Catharanthine also had cardiac effects that may contribute to the BP-lowering effects of this agent. Specifically, we observed that the injection of catharanthine caused dose-dependent reductions in HR, despite the reductions in BP that should normally cause reflex elevations of HR. The drop in HR was accompanied by a concomitant dose-dependent decrease in cardiac contractility as indicated by reductions in dP/dt max as well as the flattening of the slope of the ESPVR. Since the VOCCs in VSMCs contain the same pore-forming subunit, α1C or CaV1.2 like the majority of VOCCs in the heart, we anticipated that catharanthine might exert its effects via blockade of VOCCs (Liao et al., 2005). Indeed, we found that catharanthine blocked VOCC in ventricular cardiomyocytes and VSMC isolated from MA, but the potency of blockade was approximately 22-fold higher in the VSMCs compared with cardiomyocytes. This difference in drug potency of catharanthine on ventricular myocytes versus VSMCs is similar to that seen with other well known...
VOCC blockers such as the phenylalkylamines (like verapamil) and dihydropyridines (McDonald et al., 1994). With phenylalkylamines and dihydropyridines, blockade is voltage dependent, leading to a potency that depends strongly on the resting membrane potential of the target cells. Thus, since smooth muscle cells are relatively depolarized (resting membrane potential of approximately $-60 \text{ mV}$) compared with ventricular cardiomyocytes (resting membrane potential of approximately $85 \text{ mV}$), the potency of VOCCs is far higher for smooth muscle cells compared with ventricular cardiomyocytes. These observations are consistent with the conclusion that catharanthine inhibits VOCCs via a voltage-dependent mechanism similar to that seen with the conventional VOCC blockers. Further studies will be required to assess the molecular basis for the inhibition of VOCCs by catharanthine.

At first glance, the observation that catharanthine preferentially blocks VOCC in smooth muscle cells with a much higher sensitivity compared with ventricular myocytes suggests that the potency of the actions of catharanthine on BP might be dramatically different from its effects on the cardiac contractility. In addition, i.v. administration of catharanthine might have blocked VOCCs regulating sino-atrial (SA) and atrio-ventricular nodes leading to decreased conduction and thus lowered the HR dose dependently at concentration ranges between 0.5 and 20 mg/kg. Our in vitro data focused on ventricular myocytes; thus, it would be difficult to extrapolate the data from our in vitro findings to the in vivo setting. Future studies aimed at characterizing the VOCC blockade of SA nodal cells by catharanthine will resolve this issue. Thus, although we did not measure the Ca$^{2+}$ currents through VOCCs in SA nodal myocytes, one could anticipate that catharanthine would more potently block VOCCs in the SA node to account for its lowering of the HR.

We recently showed that the amino acid analog, L-Wee, an agent that possesses an indole ring, also exerts endothelium-independent vasodilatation of preconstricted MA (Jadhav et al., 2012). It is of interest that catharanthine is an alkaloid containing an indole ring. It is thus conceivable that some of the cardiovascular actions of catharanthine may be related to the actions of the indole ring. However, catharanthine showed a far greater potency than L-Wee on aortic rings (2 mM L-Wee versus catharanthine: IC$_{50}$ = 28–34 mM) and MA (17 mM L-Wee versus catharanthine: IC$_{50}$ = 3–6 mM). On the other hand, catharanthine and L-Wee showed a similar rank order of tissue selectivity for blocking VOCCs (MA > aorta > cardiomyocyte), suggesting a similar mechanism of action. Consistent with a role for the indole ring in, at least partially, mediating the effects of catharanthine, several previous studies have reported on the actions of indole-containing alkaloids on the cardiovascular tissues. For example, ajmalicine and hirsutine (which are also present in C. roseus) block vascular VOCCs in rat aortic rings (Yano et al., 1991).

Fig. 6. Addition of Krebs buffer at different time intervals after sustained tonic response evoked by EC$_{80-90}$ concentration of either PE (1 or 10 $\mu$M) or KCl (100 mM) depolarization in rat MA (A and B).

Fig. 7. A representative tracing that shows the inhibitory responses to the cumulative addition of catharanthine (0.5–160 $\mu$M) in parallel endothelial-denuded aortic rings constricted with either PE (1 $\mu$M) (A) or KCl (100 mM) (B). Because the time scale is compressed, and the inhibition was slow and gradual, the plateau reached at each concentration is not seen but the higher concentration of catharanthine was added only when the response reached a steady state for that particular concentration. The lower panel compares the concentration-inhibition response curves to catharanthine determined in either PE-constricted (C) or KCl-depolarized (D) aortic rings. Each data point is the mean $\pm$ S.E.M. value obtained from endothelium-denuded aortic rings isolated from $n \geq 7$ rats.
Fumigaclavine C (from Aspergillus fumigatus) blocks the tension responses to KCl in rat aortic rings (Ma et al., 2006). Geissocchizine methyl ester (from Uncariae ramulus et Uncus) induces vasodilatation in rat aortic rings (Yuzurihara et al., 2002). MB-101 (2-hydroxyacetophenone; derivative of isatin or a derivative of 1H-indole-2,3 dione) blocks the tension responses to agonists and KCl in rat aorta and cardiac papillary muscle preparations (Gabriel et al., 2011). Calindol (a 1H-indole derivative) acts as a Ca\(^{2+}\) channel antagonist in MA rings (Thakore and Ho, 2011). These observations further suggest that the indole ring in catharanthine is a factor in the ability of catharanthine to block VOCC. In this regard, a previous study concluded that the structurally related alkaloid, tetrandrine, inhibits VOCCs by binding to the phenylalkylamine [verapamil and methoxyverapamil (D-600)] binding site (King et al., 1988). Clearly, more studies are required to establish the role of drugs and alkaloids such as catharanthine that possesses an indole moiety in altering hemodynamic functions via their actions on the heart and the vasculature.

**Clinical Perspective.** Herbal extracts of *C. roseus* in concentration range (milligram doses) are reported to have a diverse range of beneficial effects (Lans, 2006; Nayak et al., 2007; Ara et al., 2009; Rasineni et al., 2010; Madagascar Periwinkle Monograph, 2011; http://naturaldatabase.therapeuticresearch.org).
Despite this, little is known regarding their effects on hemodynamics or the mechanism of action of catharanthine, a major purified compound extracted from these plants. Our results establish that catharanthine induces hypotension and reductions in HR combined with impairment of cardiac contractility. Thus, the indiscriminate use of these compounds could lead to serious cardiovascular complications that need to be considered. Indeed, the effects of catharanthine could also explain the cardiotoxicity seen in >20% of patients being treated with vinca alkaloid for cancer (Pai and Nahata, 2000). Our results provide further impetus to undertake detailed examination of the effects of other vinca alkaloids including vinflunine for their potential to contribute to cardiovascular toxicity, possibly via their effects on VSMCs and cardiac VOCCs (Pai and Nahata, 2000; Holwell et al., 2001; Kruczynski et al., 2006; Honore et al., 2008; Schutz et al., 2011; Vinflunine, 2011). In other studies (unpublished data), we have also observed that catharanthine reduces the tension responses to both ACh and KCl in isolated rat ileum and colon (IC50 5–10 μM), which can explain the ileus, constipation, and cardiac ischemia seen in cancer patients treated with vinca alkaloids (Schutz et al., 2011; Vinflunine, 2011). Finally, our studies showed that, at very low doses below those required for the induction of hypotension, catharanthine eliminated cyclical vasomotor activity and the associated calcium oscillations. It is conceivable that this action of catharanthine might be useful for the treatment of arteriolar vasospasm that is linked to stroke or other ischemic events such as angina (Inzitari and Poggesi, 2005).

**Fig. 10.** (A) Representative Ba2+ currents recorded from a single VSMC before (0 μM) and after the addition of catharanthine (3, 10, and 30 μM). Currents were elicited with voltage steps from −60 to +50 mV. (B) Current-voltage relationships of Ba2+ currents before (0 μM) and after catharanthine (3, 10, and 30 μM; n = 8 cells) or nifedipine (2 μM; n = 2 cells) treatment. (C) Current-voltage relationships of catharanthine-sensitive currents (difference between currents recorded before and after the addition of catharanthine; n = 8 VSMCs isolated from five rats).

**Fig. 11.** (A) Representative Ca2+ currents recorded from a single cardiomyocyte before and after the addition of catharanthine (100 μM, 300 μM, and 1 mM), or nifedipine (2 μM) treatment. Currents were elicited with voltage steps from −60 to +50 mV. (B) Current-voltage relationships of Ca2+ currents before (0 μM) or after the addition of either catharanthine (100 μM, 300 μM, and 1 mM; n = 3–5 cells isolated from three rats), or nifedipine (2 μM; n = 3 cells). (C) Current-voltage relationships of catharanthine-sensitive currents (difference between currents before and after the addition of catharanthine; n = 4 cells).

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