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Mechanisms underlying the biphasic effect of vitamin K_1 (phylloquinone) on arterial blood pressure

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Abstract

Phylloquinone (vitamin K_1, VK_1) is widely used therapeutically and intravenous administration of this quinone can induce hypotension. We aimed to investigate the mechanisms underlying the effects induced by VK_1 on arterial blood pressure. With this purpose a catheter was inserted into the abdominal aorta of male Wistar rats for blood pressure and heart rate recording. Bolus intravenous injection of VK_1 (0.5–20 mg kg^{-1}) produced a transient increase in blood pressure followed by a fall. Both the pressor and depressor response induced by VK_1 were dose-dependent. On the other hand, intravenous injection of VK_1 did not alter heart rate. The nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 10 and 20 mg kg^{-1}) reduced both the increase and decrease in blood pressure induced by VK_1 (5 mg kg^{-1}). On the other hand, indometacin (10 mg kg^{-1}), a non-selective cyclooxygenase inhibitor, did not alter the increase in mean arterial pressure (MAP) induced by VK_1. However, VK_1-induced fall in MAP was significantly attenuated by indometacin. We concluded that VK_1 induces a dose-dependent effect on blood pressure that consists of an acute increase followed by a more sustained decrease in MAP. The hypotension induced by VK_1 involves the activation of the nitric oxide (NO) pathway and the release of vasodilator prostanoid(s).

Introduction

Quinones represent an important class of naturally occurring compounds that are found in plants, fungi and bacteria (Kumagai et al 1998). Synthetic quinone-containing compounds are often used for therapeutic purposes, such as anti-tumour and anti-inflammatory agents (e.g. antibiotics, menadione); therefore, biological effects of those quinones have been extensively studied. Vitamin K is a group name for a number of related quinone compounds that all have a methylated naphthoquinone ring structure, and slightly differ from each other in the length and degree of saturation of their aliphatic side chain at the 3-position (Shearer 1995).

In mammals, phylloquinone (vitamin K_1, VK_1) functions as a cofactor for the endoplasmic reticulum enzyme γ-glutamyl carboxylase, which catalyses the post-translational modification of glutamate residues (Glu) into γ-carboxy glutamate (Gla) (Vermeer 1990). Well-known Gla-containing proteins are the blood coagulation factors II, VII, IX and X, which are synthesized in the liver. However, some vitamin K-dependent proteins are reported to exert direct effects on the cardiovascular system. Matrix Gla protein (MGP) is a mammalian Gla-protein expressed by human medial vascular smooth muscle cells (Spronk et al 2001), which has been suggested to be a potent inhibitor of vascular tissue calcification (Luo et al 1997; Price et al 1998). Another vitamin K-dependent Gla-protein receiving increasing attention is Gas6, which serves as a ligand for a subfamily of receptor tyrosine kinases that include Axl (Stitt et al 1995). Gas6–Axl interactions have been examined in a variety of different cell systems. The effects of Gas6–Axl interactions in the vascular tissue may be summarized as follows. In vascular smooth muscle, Gas6 was initially purified as a mediator of vascular smooth muscle proliferation (Nakano et al 1995). Later studies showed Axl up-regulation at sites of vascular injury, suggesting a role for this receptor in vascular remodelling (Melaragno et al 1998). The importance of Gas6–Axl interactions in endothelial cell physiology is demonstrated in Gas6-mediated protection of human umbilical vein endothelial cells and human pulmonary artery endothelial cells from both serum starvation and

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TNF-α-induced apoptosis (Healy et al 2001). Moreover, the protection from serum-starved apoptosis in endothelial cells is associated with Gas6–Axl interactions (D'Arcangelo et al 2000).

In addition to the above-mentioned vascular effects of VK_1, this quinone was also reported to prevent the reduction in phenylephrine-induced contraction in rat carotid arteries during hypoxia (Tirapelli et al 2002a) and to reduce acetylcholine-induced relaxation of rat carotid artery, an observation that suggested disruption of endothelial cells (Tirapelli et al 2002b, 2006).

VK_1 is used therapeutically to correct the bleeding tendency or haemorrhage associated with its deficiency and it can be given orally, intramuscularly or intravenously. Intravenous VK_1 can cause severe anaphylactic reactions, characterized by flushing and hypotension (Barash et al 1976). Although lowering of blood pressure has been associated with intravenous administration of VK_1, the physiological mechanisms underlying this response are still unknown. Thus, in this work we aimed to investigate the mechanisms underlying the hypotension induced by VK_1. With this purpose, the water-soluble analogue of VK_1, Konakion (Roche), was used as the source of VK_1.

Materials and Methods

Drugs

The following drugs were used: vitamin K_1 (Konakion, Hoffmann-La Roche, Basel, Switzerland), N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma/RBI, Natick, MA), indometacin (Calbiochem, San Diego, CA). Indometacin was prepared in Tris buffer (pH 8.4). The other drugs were dissolved in saline. The formula of Konakion utilized in this study consists of phylloquinone and Cremophor EL. The solvents did not have effect per-se on the basal blood pressure or on the pharmacological effects of VK_1 or inhibitors used in the study.

In-vivo experimental procedures

Male Wistar rats were housed under standard laboratory conditions with free access to food and water. The housing conditions and experimental protocols were in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto (University of São Paulo).

The rats were anaesthetized with urethane ($1.25\,g\,kg^{-1}$) and a catheter (a 4-cm segment of PE-10 heat-bound to a 13-cm segment of PE-50; Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta through the femoral artery for blood pressure and heart rate (HR) recording. A second catheter was implanted into the femoral vein for intravenous administration of drugs. Mean arterial pressure (MAP) and HR were recorded using an HP-7754A amplifier (Hewlett Packard, Waltham, MA) connected to a signal acquisition board (Windaq di 190; Dataq, Akron, OH). Blood pressure responses were calculated based on the average mean blood pressure calculated at the response's plateau.

Dose–response curves for VK_1 ($0.5–20\,mg\,kg^{-1}$) were obtained by bolus injection of the quinone in anaesthetized rats. The assays were carried out in a dark room since VK_1 solutions should be protected from light. Each rat received a single bolus injection of one dose of VK_1. This approach was adopted to avoid any possible influence of repeated doses of VK_1 in its effect on blood pressure. Based on the results obtained with the dose–response curves for VK_1, a dose of $5\,mg\,kg^{-1}$ was used to investigate the mechanisms underlying the effects of this quinone on blood pressure. In another set of experiments, the following inhibitors were injected intravenously 5–10 min before the administration of VK_1: L-NAME (10 or $20\,mg\,kg^{-1}$) (Elayan et al 2002) or indometacin ($10\,mg\,kg^{-1}$) (Hayashi et al 2003). The effect of VK_1 ($5\,mg\,kg^{-1}$) obtained in the absence of the inhibitors was considered as the control. VK_1-induced maximal changes (delta) in MAP were calculated from baseline MAP values (the MAP value just before the administration of VK_1). Baseline values before administration of different doses of VK_1 are reported in Table 1. Administration of L-NAME (10 or $20\,mg\,kg^{-1}$) induced an increase in MAP. The plateau observed after administration of L-NAME was considered as a new baseline. The vehicle used (saline) was systematically tested and found not to alter basal MAP or HR (data not shown).

Table 1 Mean arterial pressure (MAP) and heart rate (HR) values before (basal values) and after VK_1 intravenous administration in rats

Dose ($mg\,kg^{-1}$)	MAP (mmHg)		HR (bpm)	
	Before	After	Before	After
0.5	92 ± 5	95 ± 1/88 ± 2[a]	372 ± 27	373 ± 5/373 ± 7
1	93 ± 5	99 ± 1/80 ± 2[a]	377 ± 28	377 ± 12/378 ± 8
2.5	97 ± 4	105 ± 2/78 ± 2[a]	358 ± 15	357 ± 9/359 ± 12
5	93 ± 3	102 ± 1/74 ± 2[a]	412 ± 18	410 ± 7/416 ± 12
10	92 ± 6	105 ± 2/70 ± 3[a]	348 ± 17	344 ± 10/354 ± 10
20	95 ± 6	109 ± 1/68 ± 4[a]	351 ± 8	346 ± 9/358 ± 11

The values of MAP and HR after VK_1 are represented as the maximal effects of biphasic responses. Values are means ± s.e.m., n = 5–10. [a]$P < 0.05$, compared with the respective value before administration of VK_1. (Student's *t*-test)

Statistical analysis

Changes in MAP or HR were expressed as means ± standard error of the mean (s.e.m.). Statistical analysis was performed using one-way analysis of variance (followed by Bonferroni's comparison test) or Student's *t*-test as indicated in the text, figure and legends. Statistical analysis was performed using commercially available software (Graph Pad Prism 3.0; GraphPad Software Inc., San Diego, CA). $P < 0.05$ was considered as significant.

Results

Time-course profile of the variation in MAP induced by intravenous injection of VK_1 in anaesthetized rats

Bolus intravenous injection of VK_1 ($0.5–20\,mg\,kg^{-1}$) evoked a transient increase in blood pressure followed by a fall.

Figure 1A shows original traces of the biphasic effect induced by VK_1 on arterial blood pressure. Data presented in Table 1 illustrate the MAP and HR values before (basal values) and after administration of VK_1. All doses of VK_1 tested induced a fall in blood pressure. On the other hand, the increase in MAP induced by VK_1 was not observed with the dose of 0.5 mg kg^{-1} (Table 1). Although VK_1 evoked a transient increase in blood pressure followed by a fall, no differences in HR were observed (Table 1).

Figure 1B shows the time course of the effect induced by VK_1 (0.5–20 mg kg^{-1}) on MAP. Characteristically, VK_1 caused a dose–response change in MAP comprising of an early first phase pressor effect, which was followed by a more sustained depressor response. The doses of 5, 10 and 20 mg kg^{-1} induced a long-lasting depressor effect (time to restore to basal levels: 70 ± 7 s, 80 ± 4 s; 82 ± 5 s, respectively) when compared with the doses of 0.5, 1 and 2.5 mg kg^{-1} (time to restore to basal levels: 5 ± 3 s; 32 ± 5 s, 40 ± 6 s, respectively) ($P < 0.05$; analysis of variance followed by Bonferroni's multiple comparison test).

The effects of L-NAME and indometacin on VK_1-induced hypotension are presented in Figure 2 A, B. VK_1 at 5 mg kg^{-1} induced a maximal decrease in MAP of 13 ± 1.8 mmHg. L-NAME at 10 and 20 mg kg^{-1} significantly reduced the fall in MAP induced by VK_1 (7 ± 1.5 mmHg and 8 ± 0.5 mmHg, respectively) ($P < 0.05$; analysis of variance followed by Bonferroni's multiple comparison test). Indometacin (10 mg kg^{-1}),

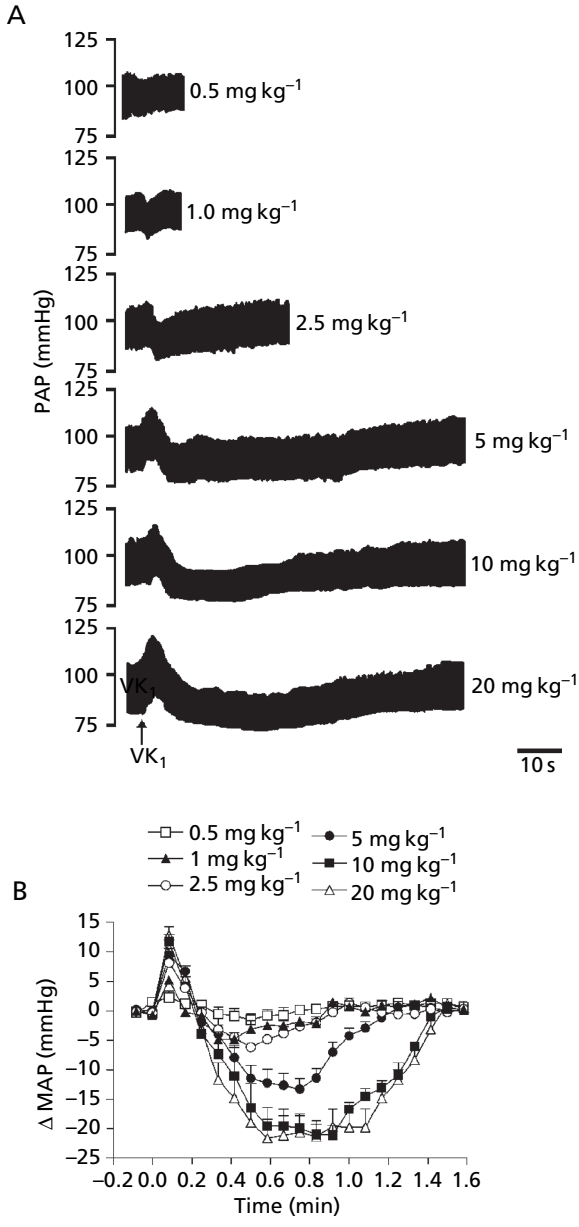

Figure 1 Original traces showing the effect of VK_1 (0.5–20 mg kg^{-1}) on pulsatile arterial blood pressure (PAP) in rats. B. Time-course profile of the variation in MAP induced by intravenous injection of VK_1 in anaesthetized rats. Bolus intravenous administrations of VK_1 (black arrow) were performed in anaesthetized male Wistar rats. The trace for each dose was determined in different rats. Each point represents the means ± s.e.m. of 5–10 rats.

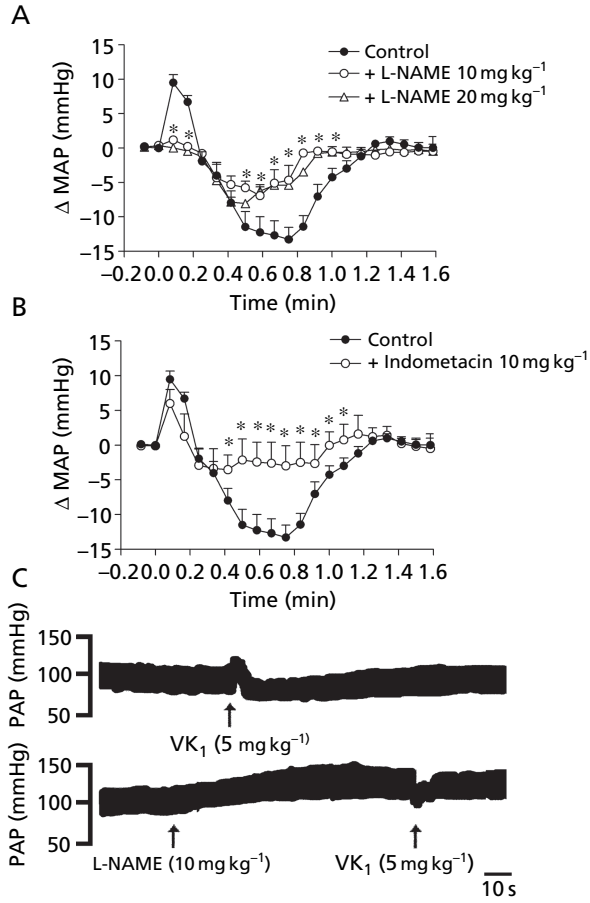

Figure 2 Effect of L-NAME (A) and indometacin (B) on the pressor and depressor response induced by VK_1 and original traces showing the effect of L-NAME (C) on pulsatile arterial blood pressure (PAP) in rats. The response induced by VK_1 (5 mg kg^{-1}) was obtained after intravenous administration (5–10 min) of the inhibitors. Results are reported as means ± s.e.m. and are representative of 5–9 rats. *$P < 0.05$, compared with control (analysis of variance followed by Bonferroni's comparison test).

a cyclooxygenase inhibitor, significantly attenuated the depressor response induced by VK_1 on MAP (3 ± 2.9 mmHg) ($P < 0.05$; analysis of variance followed by Bonferroni's multiple comparison test). Administration of L-NAME at 10 mg kg^{-1} induced an increase in MAP (139 ± 10 mmHg; n = 5) when compared with the value before the administration of the nitric oxide synthase (NOS) inhibitor (95 ± 8 mmHg, n = 5). Similarly, the administration of L-NAME at 20 mg kg^{-1} increased the MAP (153 ± 9 mmHg, n = 5) when compared with the value before the administration of the inhibitor (97 ± 7 mmHg, n = 5). On the other hand, no differences were found in MAP values before (98 ± 7 mmHg, n = 5) or after administration of indometacin (92 ± 5 mmHg, n = 5). Figure 2C shows the original trace of the effect induced by L-NAME on arterial blood pressure.

Discussion

Intravenous administration of VK_1 caused a biphasic blood pressure response, which was characterized by an initial short-lasting MAP increase followed by a longer-lasting MAP decrease component. This observation indicates that the hypotensive effect displayed by VK_1 could be more relevant than the pressor response evoked by this quinone. The VK_1-evoked MAP fall is in accordance with previous clinical reports in which intravenous administration of VK_1 caused hypotension in patients (Barash et al 1976).

L-NAME, a NOS inhibitor, reduced the pressor and depressor responses induced by VK_1, suggesting that NO plays a role in such responses. This observation supports the notion that VK_1 acts by interfering with the NO pathway to induce its effects on blood pressure, as observed previously in the isolated rat carotid (Tirapelli et al 2006). It is important to note that bolus injection of L-NAME caused elevation in baseline blood pressure indicating that constitutively available NO modulates MAP, which is in accordance with previous findings reported in the literature (Najafipour & Ferrell 1993; McLean et al 1994). Alternatively, because L-NAME treatment significantly increased baseline blood pressure (up to 50% of control values), the VK_1 pressor component could be quenched by the blood pressure elevation caused by NOS inhibition. Interestingly, L-NAME attenuated the hypotensive effect induced by VK_1, further suggesting the activation of the NO pathway in this response.

Treatment with the non-selective cyclooxygenase inhibitor indometacin did not affect VK_1-induced increase in MAP, discounting the participation of vasoconstrictor prostanoids in the pressor response induced by VK_1. However, the fall in blood pressure induced by VK_1 was significantly reduced by indometacin, suggesting that vasodilator prostanoids may play a role in this response. Thus, the hypotension induced by VK_1 would involve the release of prostacyclin, since it is well known that this arachidonic acid metabolite causes vascular relaxation (Bunting et al 1976; Dusting et al 1977) and hypotension (Bayorh et al 2002). Increasing evidence suggests the existence of a cross talk between NO and prostaglandins biosynthetic pathways (Mollace et al 2005). In this line, NO was reported to activate the enzyme cyclooxygenase and to increase the synthesis of prostaglandins such as prostacyclin (Salvemini et al 1993, 1996). On the other hand, the metabolites generated by cyclooxygenases may also interfere with the biosynthesis of NO (Chen et al 1997). Thus there is consistent evidence showing the synergy between the vascular action of NO and prostaglandins (Salvemini et al 1993). In this line, the production of vasodilator prostaglandins would be crucial to the vasodilator action displayed by NO. Thus, considering these results obtained with indometacin and L-NAME, we could suggest that the hypotension induced by VK_1 involves the generation of NO and vasodilator prostaglandins, which would be acting synergistically to mediate the hypotension induced by VK_1.

VK_1 has several clinical applications and different doses of this quinone are usually administered orally, intramuscularly or intravenously. In this study, doses of VK_1 ranging from 0.5 to 20 mg kg^{-1} were used. These doses were based on previous studies conducted in rats and rabbits in which VK_1 was administered intravenously at doses of 1 mg kg^{-1} (Wilson & Park 1984) or 10 mg kg^{-1} (Winn et al 1988) to analyse the pharmacokinetics of this quinone. Although the doses used in our investigation are higher than those used therapeutically, the lower doses of 0.5 and 1 mg kg^{-1} are well within the range of VK_1 used in some studies from the literature carried out in man. Raith et al (2000) used doses of VK_1 ranging from 0.2 to 0.7 mg kg^{-1} to verify the plasma levels of this quinone after intravenous administration of Konakion. Similarly, a dose of 1 mg was intravenously administered to infants with birth weight ranging from 1.7 to 5 kg evaluation of serum concentrations of VK_1 (Pereira et al 2003).

In addition to its effects on blood pressure, VK_1 was also reported to exert direct vascular effects. Previously, we described that in the isolated rat carotid, VK_1 induces superoxide anion generation, which in turn is involved in the increased response of this artery to phenylephrine, a selective α_1-receptor agonist (Tirapelli et al 2006). It is important to note that superoxide anion plays a critical role in cellular toxicity and tissue injury (Cadenas 1989). Superoxide anions may not only induce cytotoxicity in endothelial cells but also alter endothelial function by modification of the NO pathway and finally cause vascular injury. Thus, we must be aware of the potential deleterious effects of repeated administration of VK_1 since many experimental data have shown that an increased exposure of vascular tissue to superoxide anions is associated with endothelial dysfunction and many vascular diseases such as atherosclerosis and hypertension (Hammond et al 1985; Halliwell 1987).

Conclusion

To the best of our knowledge, this is the first study to evaluate the mechanisms underlying the effects of VK_1 on arterial blood pressure in-vivo. We conclude that VK_1 induces a biphasic effect on blood pressure that consists of an acute increase followed by a more sustained decrease in MAP. The hypotension induced by VK_1 involves the activation of the NO pathway and the release of vasodilator prostanoid(s).

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