Vulgarenol Effect on Coronary Vascular Resistance

del Valle Mondragón L., Tenorio López F.A., Torres Narváez J.C., Zarco Olvera G., Pastelín Hernández G.

Departamento de Farmacología,
Instituto Nacional de Cardiología "Ignacio Chávez".
Tlalpan, México D. F., México.

ABSTRACT
Introduction: Magnolia grandiflora was firstly typified by Linneo as Magnolia grandiflora (L.) in 1759. It is an ever-green tree introduced by the Spanish during the conquer and is now widely distributed in America. Ethnomedically, it has been previously reported that M. grandiflora extracts have been used since ancient times to treat cardiac pathologies, a practice that remains to this day.

Materials and methods: Vulgarenol, a sesquiterpene isolated and purified from Magnolia grandiflora flowers petals extracts by high-performance liquid chromatography (HPLC), was pharmacologically evaluated on isolated and perfused guinea pigs hearts according to Langendorff. In another experimental group, hypertension was induced by chronic blockade of nitric oxide synthesis by administrating Nω-nitro-L-arginine methyl ester (70 mg/kg, i.p.). Under these conditions, coronary vascular resistance, nitric oxide production and cyclic guanosine monophosphate release were evaluated.

Results: Vulgarenol decreases coronary vascular resistance in isolated and perfused guinea pigs hearts according to Langendorff. Our results suggest that vulgarenol pharmacological action ongoes with statistically significant increases in nitric oxide and cyclic guanosine monophosphate production, which were inhibited by the blocking effect of antagonists and inhibitors such as gadolinium (III) chloride, Nω-nitro-L-arginine methyl ester and 1H-[1,2,4] oxadiazolo [4,2-a] quinoxalin-1-one. In the hypertensive animals, it was observed that vulgarenol partially reverts hypertension induced by chronic blockade of nitric oxide synthesis. Additionally, drugs such as pentoxyphylline and milrinone potentiate the pharmacological effect induced by vulgarenol. Such effects suppose a calcium-dependent process, along with the possible participation of the constitutive and inducible nitric oxide synthases, increasing cyclic guanosine monophosphate production by means of phosphodiesterase III inhibition.

Conclusion: Our results support the fact that vulgarenol-induced vasorelaxation is mediated through the nitric oxide/soluble guanylyl cyclase pathway.

INTRODUCTION

Magnolia grandiflora was typified by Linneo in 1759 [1]. It is an evergreen tree, with a height of 3 to 20 m, with a dense and dark tip, with a deep grey-colored and short log. Leaves are alternate, light-green colored, observed in 10-20 cm handfuls, which are 7-10 cm wide. Flowers are perfumed, white-colored, found solitaire, and appear on the tree from May to July. It was introduced by the Spanish during the conquer, spreading into North and South America [2,3].

Ethnobotanic-historic studies carried out in Mexico [3,4] point that since Colony times, flowers and leaves from this tree were used by our ancestors in the ethnomedical practice to treat cardiac pathologies accompanied by throbbing and respiratory problems. Nowadays, both their vasodilator and positive inotropic effects are being studied [5,6].

On the other hand, vascular tone is mainly maintained by the precise balance of endogenous/constrictor...
Nitric oxide (NO) has been regarded as the most important endogenous vasodilator, which is affected by nitric oxide synthase competitive inhibitors, such as Nω-L-nitro-arginine methyl ester (L-NAME), which inhibits NO synthesis by the chronic blockade of NOS-1 and NOS-3 isoforms, increasing by such in vivo blood pressure, and inhibiting NOS-2 isoform expression in ischemia-reperfusion models as well [8,9]. Once it has been synthesized, NO diffuses to adjacent cells, activating by such an enzyme known as soluble guanylyl cyclase (sGC), causing an infold in cyclic guanosine monophosphate (cGMP) production. This increase, activates in turn a cGMP-dependent kinase, generating by such, vasodilation in smooth muscle cells [10,11]. This mechanism is known as the NO/sGC pathway, which can be selectively inhibited by 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ), generating hypertension by the uncoupling of the endogenous vasodilator machinery [12-16].

Phosphodiesterases selective inhibition is the primary action mechanism of drugs such as amrinone, milrinone, pentoxiphylline, aminophylline and sildenafil, among many others [17], along with some natural products such as terpenoids and flavonoids [18]. Phosphodiesterase inhibition produces a relaxation of venous and arterial smooth muscle, which is Ca^2+ increases-mediated, with infolds cGMP, via NO [18].

It has been observed that most NO-induced vasodilation pathways are Ca^2+ -dependent, therefore, either in vitro and in vivo studies show that unspecific blockade of this ion with 3 m M gadolinium disrupts intracellular Ca^2+ influx, inhibiting by such the endothelium-dependent vasodilation, which is promoted by intracellular Ca^2+ and by the NO/sGC pathway [17-19].

MATERIALS AND METHODS
Natural product
Vulgarenol was isolated and purified by high-performance liquid chromatography (HPLC), from aqueous extracts from Magnolia grandiflora flowers petals, as previously described [20].

Drugs
Isosorbide dinitrate (ICN Biomedicals, Aurora, Ohio, USA).
Verapamil hydrochloride (Sigma Chemical Co., St. Louis, Missouri, USA).
Sildenafil citrate (SynFine Research, Richmond Hill, Ontario, Canada).
Pentoxiphylline (ICN Biomedicals, Aurora, Ohio, USA).
Gadolinium (III) chloride (Sigma Chemical Co., St. Louis, Missouri, USA).
Nω-nitro- L-arginine methyl ester (L-NAME) (Sigma Chemical Co., St. Louis, Missouri, USA).
1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ) (Sigma Chemical Co., St. Louis Missouri, USA).

Animals
For this study, male guinea pigs (900-950 g) were used. They were kept under normal light and temperature conditions, with water and fed with a standard diet (LabDiet 5026, PMI Nutrition International) ad libitum. All animal experimentation had adhered to ethical guidelines for animal experimentation approved by the Ethics Committee of the Instituto Nacional de Cardiología "Ignacio Chávez".

Vasodilator activity of vulgarenol
Vasodilator activity of vulgarenol was assayed on the Langendorff isolated and perfused heart model [21]. Under this model, coronary vascular resistance (CVR) was indirectly recorded by registering perfusion pressure by inserting a hydropneumatic transductor (Glixo R25), at the right and left ostium located at the ascendant aorta. Pressure variations were recorded in a polygraph (Grass 79D, Grass Instrument Co., Quincy, Massachusetts, USA). Cardiac frequency was kept constant at 1 Hz by means of a ventricular epicardic pacemaker (Grass SIU5, Grass Instruments Co., Quincy, Massachusetts, USA). Drugs were administered by a dosificator (Hamilton) adjacent to the perfusion cannula.

NO quantification
NO production was evaluated on the perfusion liquid by the method of Kelm and Schrader [22], under UV-
Vis spectrometry by extinction coefficient (401-411 nm) at room temperature. All determinations were performed on a double-beam UV-Vis spectrometer (SLM-AMINCO DW2000, SLM Instruments, Urbana, Illinois, USA).

**cGMP assay**
Left ventricular segments were quickly frozen on liquid nitrogen. These tissue samples were homogenized in cold (0 to 4 °C) with a tissue homogenator (Polytron, Daigger, Vernon Hills, Illinois, USA), previously suspended in a 5% trichloroacetic acid solution. Homogenates were centrifuged at 1500 rpm/per gram of tissue during 10 minutes at 4 °C. Supernatants were collected and were washed with 5 volumes of ether-saturated water. Later on, ether was evaporated under nitrogen steam. cGMP content was evaluated as indicated by the kit maker (Cyclic GMP EIA kit, Cayman Chemicals, Ann Arbor, Michigan USA). Quantification was determined at 415 nm using a spectrometer (Cary 4000, Varian, Mulgrave, Victoria, Australia). Assay sensibility was 1.0 fmole/mg of tissue. Intra-assay variations were lesser than 2.5%.

**STATISTICS**
As statistical analysis, one-way ANOVA followed by Student’s *t*-test for paired data [23], with n=10 per group and *P*<0.05.

**RESULTS AND DISCUSSION**
*In vitro* performed studies showed that vulgarenol produces a dose-dependent vasodilator effect because it decreases, when compared to control group, CVR, when also compared with other vasodilators such as isosorbide dinitrate, verapamil and sildenafil (Figure 1).

Vulgarenol causes a greater statistically significant vasodilation than the drugs used as positive controls, and due to the fact that is a natural product, chemically identified as a sesquiterpene, it is very likely that, as observed with other terpenoids and flavonoids, this vasodilator effect could be mediated by the NO/sGC pathway [24-27], which can be inferred by increases in NO and cGMP as well (Figures 2 and 6). To corroborate such facts, in a first attempt, we blocked stretch-activated ion channels with 3 mM gadolinium [19]. Once the blockade is observed, an increase in CVR is observed and an decrease in NO (Figure 2), we continuously infused 5 mM vulgarenol, without interrupting gadolinium administration, and it can be observed that vulgarenol is unable to reverse gadolinium-induced hypertension. No statistically

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*Figure 1: Vulgarenol effect on coronary vascular resistance (CVR) compared to isosorbide, verapamil and sildenafil. Data are expressed as mean ± MES; n=10 per group; *P*<0.05 vs. control group.*

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significant changes in CVR and NO were observed, whereas cGMP production was discrete (Figures 2 and 6).

**Figure 2:** 5 mM vulgarenol effect on coronary vascular resistance (CVR) and nitric oxide (NO) release in the presence of 3 mM gadolinium (III) chloride. Data are expressed as mean ± MES; n=10 per group; *P<0.05 vs. control group.

**Figure 3:** 5 mM vulgarenol effect on coronary vascular resistance (CVR) and nitric oxide (NO) release in the presence of 100 mM L-NAME. Data are expressed as mean ± MES; n=10 per group; *P<0.05 vs. control group.
Figure 4: 5 mM vulgarenol effect on coronary vascular resistance (CVR) and nitric oxide (NO) release in the presence of 10 mM ODQ. Data are expressed as mean ± MES; n=10 per group; *P<0.05 vs. control group.

Figure 5: 5 mM vulgarenol effect on coronary vascular resistance (CVR) and nitric oxide (NO) in the presence of 15 mM pentoxyphylline. Data are expressed as mean ± MES; n=10 per group; *P<0.05 vs. control group.
Alternatively, NOS were blocked with a continuous infusion of 100 m M L -NAME. Once unspecific inhibition is produced (as CVR increased and NO production decreased) (Figure 3), we continuously infused, without stopping L -NAME infusion, 5 m M vulgarenol, which can't revert L -NAME vasoconstrictor effect (Figure 3), due to the fact that, in L -NAME presence, vulgarenol does not seem to stimulate cGMP production (Figure 6).

Further on, NO/sGC pathway was blocked [27-29], continuously infusing 10 m M ODQ, which is selective inhibitor of this pathway [28,29]. Once this pathway was blocked, as it can be deduced from increases in CVR and decreases in NO release (Figure 4), 5 m M vulgarenol was continuously infused, without interrupting ODQ administration. The observed results showed that vulgarenol is again unable to reverse ODQ blocking effect. cGMP maintained without statistically significant changes (Figure 6).

Once confirmed that the vulgarenol showed a NO/sGC-dependent vasodilation, we decided to evaluate if, as observed with flavonoids and terpenoids, vulgarenol is a phosphodiesterase-3 inhibitor [29,31]. For such, 15 m M pentoxyphylline was administered. Once CVR decreased and NO release increased (Figure 5), 5 m M vulgarenol was infused, observating a synergistic effect, which correlates with either NO and cGMP release increases (Figures 5 and 6).

CONCLUSION
Vasodilator effect elicited by vulgarenol is a calcium-dependent process, with a possible participation of the endothelial and inducible nitric oxide synthases, increasing by such cGMP. This effect could also be mediated by phosphodiesterase-3 inhibition, which can also indicate that NO/sGC actively participates in this pharmacological effect.

BIBLIOGRAPHY

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