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Review Article

Targeting Nitric Oxide with Natural Derived Compounds as a Therapeutic Strategy in Vascular Diseases

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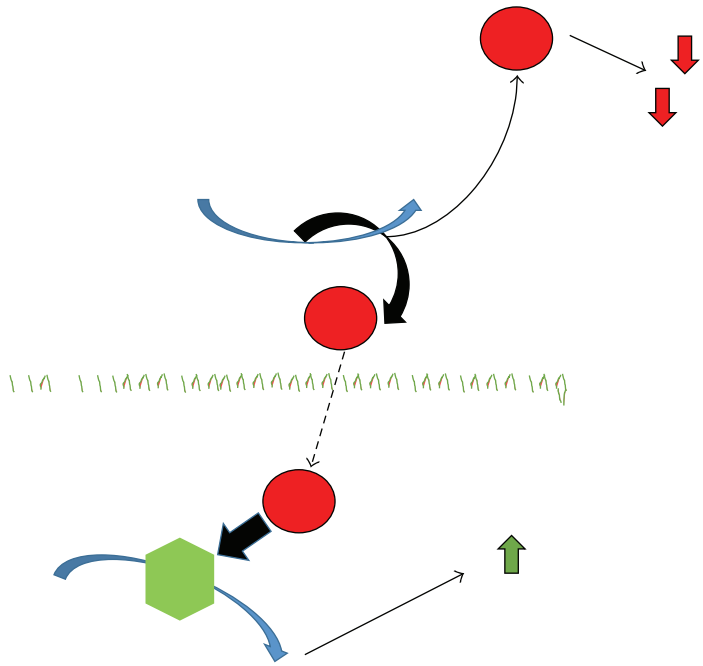
Within the family of endogenous gasotransmitters, nitric oxide (NO) is the smallest gaseous intercellular messenger involved in the modulation of several processes, such as blood ow and platelet aggregation control, essential to maintain vascular homeostasis. NO is produced by nitric oxide synthases (NOS) and its e ects are mediated by cGMP-dependent or cGMP-independent mechanisms. Growing evidence suggests a crosstalk between NO signaling and the occurrence of oxidative stress in the onset and progression of vascular diseases, such as hypertension, heart failure, ischemia, and stroke. For these reasons, NO is considered as an emerging molecular target for developing therapeutic strategies for cardio- and cerebrovascular pathologies. Several natural derived compounds, such as polyphenols, are now proposed as modulators of NO-mediated pathways. e aim of this review is to highlight the experimental evidence on the involvement of nitric oxide in vascular homeostasis focusing on the therapeutic potential of targeting NO with some natural compounds in patients with vascular diseases.

1. Introduction

Since , when nitric oxide (NO) was nominated •molecule of the yearŽ [,], it continues to attract the interest of the scienti c community. NO is the smallest gasotransmitter, recognized as an ubiquitous intercellular messenger; it is produced by three isoforms of NO synthases (NOS): endothelial NOS (eNOS) [ ], neuronal NOS (nNOS) [ ], and inducible NOS (iNOS) [ ] and mitochondrial NOS (mtNOS) [ ]. All NOS isozymes utilize L-arginine and oxygen and the reduced form of nicotinamide-adenine-dinucleotide phosphate (NADPH) as substrates and R- , , , -tetrahydro-L-biopterin (BH_4) as essential cofactor to generate NO and L-citrulline [,]. en, the main downstream signaling pathway carried out by the NO is the activation of soluble guanylyl cyclase (sGC), which in turn generates cyclic guanosine monophosphate (cGMP) [ ] (Figure).

In the vascular system NO modulates blood ow [ ], vascular tone [ ], and platelet aggregation [ ] exerting antihypertensive, antithrombotic, and atherosclerotic e ects. It is also involved in the stimulation of the endothelial progenitor cells (EPCs) and proliferation of the smooth muscle cells (SMCs) [ ]. erefore, an impairment in the NO signaling is associated with the onset and perpetuation of the main clinical condition associated to cardiovascular diseases (CVDs) including endothelial dysfunction [ ].

Given this premise, it is reasonable to consider NO as a therapeutic target for CVDs. Indeed, several approaches have been proposed to modulate NO pathways while preserving its physiological role [ ]. From one side, the strategy consists in enhancing NO bioavailability, principally acting on NOS cofactors or avoiding NO breakdown; from the other side, di erent drugs act on the NO downstream signaling targets [ ].



$O_2^{\bullet-}$ reduction, thereby leading to decrease in blood pressure [ ]. Moreover, soy iso avones has also been demonstrated to improve the NO metabolism in carotid and cerebral rat arteries [ ] as well as to enhance eNOS mRNA expression [ ].

NO-mediated antihypertensive e ects were also reported in rats a er administration of other soy iso avones, such as glucosyl hesperidin [ ]. Yamamoto et al. found that the hypotensive e ects of this natural compound were associated with reduction of oxidative stress and improvement of the NO metabolism [ ]. In this regard, hesperidin was found to signi cantly prevent endothelial damage and leucocytes adhesion in animal models of ischemia reperfusion. Concomitantly, an increase of NO bioavailability and a reduction of in ammatory molecules which contribute to ameliorate edema and other symptoms of venous diseases have been reported [ ].

Polyphenol-rich cocoa extracts have been demonstrated to reduce blood pressure in spontaneously hypertensive rats [ ] and, similarly, in hypertensive patients, as well in healthy subjects, the intake of black cocoa extracts has been reported to reduce blood pressure and improve endothelial function through increase of the NO bioavailability […]. Moreover, in patients with high cardiovascular risk it was showed that the administration of two di erent diets, one rich in polyphenols deriving from extra virgin olive oil and another rich in nuts, was shown to reduce systolic and diastolic pressure concomitantly with an increase of the NO plasma levels [ ].

. . Red Wine Polyphenols and NO Pathways. Red wine is one of the main sources of the natural polyphenols. As mentioned above, epidemiological studies have suggested that the high consumption of red wine correlates with a reduction of the CVDs risk factors. e evidence corroborating vascular e ects of red wine polyphenols (RWPs), as well as grape seed extracts (GSEs) and grape juice polyphenols (GJPs), is the induction of NO-dependent relaxation in isolate arteries and the activation of NO signaling pathways in endothelial cells […]. Leikert et al. found that RWPs enhanced eNOS expression and release of NO in human endothelial cells [ ]. In the same way, NO production and intracellular Ca^{2+} release have been shown in bovine endothelial cells treated with RWPs [ ] and an increase of eNOS and Akt phosphorylation were also reported in endothelial cells exposed to GSEs [ ]. Similar eNOS activation was also demonstrated in isolated arteries. For example, in porcine coronary arteries Madeira et al. showed endothelium relaxation induced by GSEs via Akt/eNOS phosphorylation [ ], and also in isolated porcine coronary arteries, RWPs were found to enhance phosphorylation of eNOS at Ser , resulting in the increase of the NO production [ ]. Interestingly, in rat femoral arteries, RWPs were shown to induce vasodilatation and to increase the NO levels in a concentration-dependent manner [ ]. Moreover, RWPs were demonstrated in rat aorta to enhance NO bioavailability and to increase intracellular Ca^{2+} and cGMP concentrations [,].

Several molecular mechanisms have been proposed to explain in both animal models and humans the bene cial e ects of the RWPs in vascular physiology. In this regard, Berrátová et al. in hypertensive NO de cient rats showed that RWPs restored endothelial functions thanks to a reduction of blood pressure induced by increased eNOS activity in the le ventricle and aorta [ ].

Similarly, in salt-induced hypertensive rats, RWPs were shown to improve vascular physiology by inhibiting NADPH oxidase [ ]. e inhibition of NADPH oxidase was also reported in Ang II hypertensive rats treated with RWPs in which a reduction of superoxide anions level occurred concomitantly with restoration of the NO bioavailability [ ]. RWPs have been demonstrated to exert protective e ects also in animal models of ischemia and atherosclerosis. For example, in ischemic rats, RWPs were shown to reduce the angiogenic process [ ], and, in hypercholesterolemic mice, Napoli et al. showed that low doses of RWPs reduced atherosclerosis by eNOS activation [ ]. Interestingly, with an in vitro model of human atherosclerosis, Magrone et al. have reported enhanced production of the NO, a er administration of red wine. e authors tested some red wines for their ability to trigger NO production from human healthy peripheral blood mononuclear cells, nding that avonoids and resveratrol, abundant in the red wine, once absorbed at intestinal level and entered into circulation, induced monocytes to produce the NO [ ].

Few clinical trials have planned with the aim to investigate the e ects of a dietary regimen based on moderate consumption of wine about NO related improvement in vascular physiology in both healthy patients and patients with high risk of CVDs. For example, in healthy subjects, an oral supplementation of grape juice was found to inhibit platelet aggregation with decreased production of superoxide and enhanced NO levels [,]. Moreover, besides its antithrombotic activity, red wine has also been suggested to exert cardiovascular protective e ects by enhancing circulating endothelial progenitor cells thanks to a mechanism involving an increase of the NO bioavailability, as reported in studies performed in healthy individuals by Huang et al. [ ]. In addition, red wine consumption has been shown to signi cantly decrease blood pressure and enhance plasma NO levels in hypertensive patients [ ]. Interestingly, Karatzi et al. demonstrated that in smokers a consumption of red wine counterbalanced the endothelial dysfunction caused by oxidative stress induced by cigarettes smoke, in a pathway probably mediated by NO [ ].

. . Resveratrol and NO Pathways. Among the RWPs, resveratrol (RSV) is one of the best characterized members. It has been used in the Indian medical herb named •DarakchasavaŽ from about years ago and the clinical e ects described in the past for •DarakchasavaŽ are the same attributed to RSV today [ ]. RSV was rstly described for its antitumorigenic properties [ ]; it is present especially in grape skin and red wine, but also in peanuts, pistachios, and pine trees [ ]. e interest of the scienti c community for RSV derives from the observation that its administration mimics the e ects of calorie restriction, a tool widely recognized to prevent the endothelial dysfunction, thereby attenuating atherosclerosis,

hypertension, diabetes, and CVDs risk factors and aging-associated diseases in general […]. anks to some experiments conducted in vitro in endothelial cells, RSV has been shown to regulate several target molecules, such as the NAD^+-dependent deacetylases named sirtuins, acting at transcriptional and posttranscriptional levels […].

Although the studies underlining the vascular protective e ects exerted by RSV did not study the involvement of the NO signaling [,], several ndings, obtained in animal models of CVDs, have proposed the NO as the main downstream target mediating such e ects. For example, Xia et al. demonstrated in ApoE de cient mice that RSV was able to modulate the oxidative stress responsible for atherosclerosis. From one side, NADPH oxidases were downregulated; from the other side superoxide dismutases (SOD) were upregulated. Moreover, oxidation of BH_4 was found to be reduced, attenuating the increase of eNOS uncoupling levels [ ]. Other bene cial e ects were shown in many di erent clinical settings reinforcing the idea that RSV could be considered an optimal therapeutic strategy against CVDs. For example, in hypercholesterolemic rabbits, RSV improved endothelial function in parallel with an increase of NO plasma levels [ ]. In addition, RSV has been suggested to contrast the endothelial dysfunction correlated with metabolic syndromes. In this regard, in endothelial cells RSV was demonstrated to suppress superoxide generation and to activate eNOS through phosphorylation at Ser thereby increasing the NO generation [ ]. In aortas of diabetic mice, RSV restored vasodilatation by enhancing eNOS activity and inhibiting the tumor necrosis factor- (TNF -) induced activation of NADPH oxidase [ ]. In the same way, a treatment in rats with RSV has been showed to increase muscle microvascular recruitment via an NO-dependent mechanism blocked by TNF [ ]. Also, RSV was shown to reduce blood pressure in obese rats and to enhance the expression of eNOS via AMPK and reduction of TNF in adipose tissue [ ]. Similarly, in rats fed with high fructose diet, RSV decreased blood pressure via AMPK-Akt-NOS pathway [ ]. Interestingly, in the myocardium of diabetic mice, RSV reduced Cav- expression, which in turn contributes to enhance eNOS activity [ ], and the same e ects on Cav- expression were found in hypercholesterolemic rats [ ].

Furthermore, RSV was shown to protect heart from ischemic reperfusion injury. Hattori et al. demonstrated that RSV reduced infarct size in rat hearts by enhancing iNOS expression [ ]. e cardioprotective e ects of the RSV has also been showed in spontaneously and angiotensin Ang II-induced hypertensive rats, in which RSV contributes to the upregulation of the eNOS activity and reduction of pressure and cardiac hypertrophy [ ]. Moreover, the antihypertensive e ect of the RSV was also shown to be mediated by the attenuation of eNOS uncoupling via reduction of L-arginine levels and oxidative stress [ ].

e antithrombotic activity of the RSV has been also reported in human platelets. Gresele et al. showed that RSV stimulated platelet NO production through inhibition of p MAPK, NADPH oxidases, and superoxide formation, thus decreasing peroxynitrite accumulation [ ].

RSV was also shown to mobilize endothelial progenitor cells in a NO-dependent manner, thus contributing to repairing the damage occurring in vessels a er ischemic injuries as].

In the arteries of patients with hypertension and dyslipidemia, Carrizzo et al. characterized many of the downstream e ectors of the RSV-dependent NO generation. e authors found an enhanced vasodilatation of arteries due to the activation of AMPK and reduction of eNOS uncoupling via increasing levels of BH_4 and, in the same study, RSV was found to reduce vascular oxidative stress trough upregulation of manganese superoxide dismutase in a pathway mediate by nuclear factor erythroid-derived -like [ ].

Some authors have also suggested the potential therapeutic use of RSV for the prevention of stroke; for example, in rat models of stroke, RSV reduced brain damage in a NO-dependent manner [ ]. Similarly, in rats subjected to focal cerebral ischemia Tsai et al. provided the evidence that RSV might enhance plasma levels of the NO and upregulate eNOS expression while it might downregulate iNOS expression and that these e ects were abolished by the coadministration of selective NOS inhibitors [ ].

8. Bioavailability of Polyphenols

Although the use of the polyphenols represents a promising tool for increasing the NO production and activity against CVDs, one of the biggest challenges for their employ in the clinical practice is to enhance their low bioavailability. In this regard, it has been shown that when orally administered, polyphenols concentration appears not to be su cient to ensure therapeutic e ects [ ]. For example, the plasmatic levels of the resveratrol from dietary intake are o en undetectable or very low when compared with the concentrations employed duringin vivo and in vitro experiments [ ]. Similarly, the pharmacological properties of curcumin are drastically restricted mainly because of its low water solubility and absorption from the gut, short half-life, and extremely poor bioavailability.

To overcome such problems, one of the best approach could be developing new pharmaceutical formulations, for example, polyphenols conjugated with cyclodextrins, or encapsulated in nanoparticles (NP), such as poly(lactic-co-glycolic acid) (PLGA) based NP or liposomes. In this regard, many of these formulations have been demonstrated to improve solubility, systemic half-life, resistance to metabolic degradation, and ultimately the bioavailability of the polyphenolic compounds in order to potentiate their biological activities [,]. However, while the di erences between polyphenols monoadministered or administered in encapsulated formulations have been extensively studied for what concerns the polyphenols antioxidant and anticancer properties, no experiments have been carried out on the e ects of these formulations on the NO metabolism.

9. Conclusion

Targeting the gasotransmitter NO is becoming a new challenge in cardiovascular medicine. We here reviewed some

of the experimental evidences that have indicated several natural compounds as suitable activators of the NO signaling pathways.

It is necessary to remark that for most of them the molecular mechanism, as well as the precise concentration to obtain bene cial e ects, especially because of their low bioavailability remains to be determined. Nevertheless, these agents, mainly the polyphenols, doubtless possess a great therapeutic potential above all when you consider that the available drugs, although e ective, did not act exclusively on the NO pathways o en causing deleterious side e ects. Moreover, most of the investigations on the natural compounds have involved in vitro studies; thus it is di cult to draw de nite conclusions about their therapeutic usefulness.

Although accumulating evidence suggests that the polyphenols exert bene cial e ects against vascular diseases by restoring the impairment of the NO production and/or bioavailability, much remains to be clari ed. Doubtless, many gaps must be lled in understanding the complex chemistry, biochemistry, and molecular biology of such natural agents in order to introduce such NO signaling modulators in the clinical practice.

Competing Interests

e authors declare that they have no competing interests.

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