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From Field to Health: A Simple Way To Increase the Nutraceutical Content of Grape As Shown by NO-Dependent Vascular Relaxation

FRANCESCA FUMAGALLI,[†,‡] MARA ROSSONI,[†,§] MARCELLO IRITI,[#] ANTONIO DI GENNARO,[‡] FRANCO FAORO,[⊥] EMANUELE BORRONI,[‡] MICHELE BORGO,[||] ATTILIO SCIENZA,[§] ANGELO SALA,[‡] AND GIANCARLO FOLCO*[,‡]

Dipartimento di Scienze Farmacologiche, Dipartimento di Produzione Vegetale, and Istituto di Patologia Vegetale, Università di Milano, Italy; Istituto di Virologia Vegetale, CNR, and A.C.R. Istituto Sperimentale per la Viticoltura, Conegliano Veneto, Treviso, Italy

Polyphenolic grapevine components involved in plant resistance against pathogens possess various pharmacological properties that include nitric oxide (NO)-dependent vasodilation and anti-inflammatory and free radical scavenging activities, which may explain the protective effect of moderate red wine consumption against cardiovascular disease. The aim of this work was (a) to verify the possibility that preharvest treatments of grapevine with a plant activator, benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), could lead to an enriched nutraceutical potential of wine and (b) to characterize the profile of metabolites responsible for pharmacological activity. Plant spraying at the end of veraison, with a water suspension of BTH (0.3 mM), led to increased whole anthocyanin content as confirmed by HPLC comparative analysis. Extracts from berry skins of BTH-treated grapevines caused NO-dependent vasorelaxation, with a concentration−response curve that was significantly shifted to the left of the control non-BTH-treated curve. Moreover, 1:1000 dilutions of berry extracts from BTH-treated plants significantly increased basal production of guanosine 3′,5′-cyclic monophosphate (cGMP) in human vascular endothelial cells when compared to the corresponding extracts of untreated plants. These results show that BTH treatment increases anthocyanin content of grape extracts, as well as their ability to induce NO-mediated vasoprotection. No increase of anthocyanin content was observed in the wine extracts from BTH-treated vines. It is concluded that BTH treatment could be exploited to increase the nutraceutical potential of grapes.

KEYWORDS: Nitric oxide; vasodilation; plant activator; polyphenols; grape pharmaconutrients

INTRODUCTION

Epidemiological studies support an association between a moderate wine intake and lowering of coronary heart disease (CHD) risk and, although some cardioprotective effects may be due to ethanol intake, significant research has focused on the potential protective non-lipoprotein-altering effects of substances unique to wine. In fact, it is reasonable to speculate that the cardioprotective effects of wine observed in French and other populations may be mainly ascribed to its polyphenolic components (*1*). Flavonoids and stilbenes, also referred to as polyphenolics, are present in the leaves and berry skin of most vine cultivars; among flavonoids, anthocyanins are the most prominent pigments in grape skin, whereas resveratrol represents the major end-product of the stilbene-synthase pathway (*2*). In the plant kingdom, anthocyanins and resveratrol are classified as phytoalexins; they are involved in inducible resistance against pathogens, and their concentrations in plant tissue may increase markedly in response to microbial challenge or chemical treatment (*3*). Recent evidence indicates that anthocyanins and resveratrol possess a broad array of pharmacological properties that include vasodilation as well as anti-inflammatory and free radical scavenging activities. In particular, the enhanced generation of nitric oxide (NO), a platelet inhibitor and a powerful vasodilator, caused by polyphenolic red wine components in vitro and in vivo, constitutes an important modulatory component of hemostatic function and may represent a clinically relevant mechanism to explain the protective effect of moderate red wine consumption on cardiovascular disease in man (*4*, *5*).

* Address correspondence to this author at the Dipartimento di Scienze Farmacologiche, Via Balzaretti 9, 20133 Milano, Italy (telephone +390250318308; fax +390250318385; e-mail giancarlo.folco@unimi.it).
[†] Co-first authors.
[‡] Dipartimento di Scienze Farmacologiche, Università di Milano.
[§] Dipartimento di Produzione Vegetale, Università di Milano.
[#] Istituto di Patologia Vegetale, Università di Milano.
[⊥] Istituto di Virologia Vegetale, CNR.
[||] A.C.R. Istituto Sperimentale per la Viticoltura.

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We provide here evidence that preharvest multiple treatments of grapevine with the plant activator benzothiadiazole (BTH), also reported as acibenzolar-*S*-methyl [*S*-methyl benzo-(1,2,3)-thiadiazole-7-carbothioate, CGA 245-704], increase the content of two important classes of phytoalexins, as previously reported by Iriti and colleagues (*6*, *7*), as well as the postharvest nutraceutical potential of grapevine berry extracts, but not of wine constituents, via increased nitric oxide production in blood vessels in vitro.

MATERIALS AND METHODS

Plant Materials and Treatments. Plant (*Vitis vinifera* cv. Merlot) treatments were carried out in an experimental vineyard at Conegliano Veneto (Treviso, Italy). Plants were sprayed on the first, fourth, and seventh day of the last week of August 2003, at the end of veraison, with a water suspension of BTH (trade name Bion, Syngenta, Basel, Switzerland) at the concentration of 0.3 mM, prepared from a wettable formulation containing 50% (w/w) active ingredient (ai). This concentration has been selected on the basis of results obtained in a previous study on bean plants (*8*). Control plants were sprayed with a water suspension of wettable powder without ai. Mature clusters were collected from treated and untreated plants at harvest, that is, 4 weeks after BTH treatments, for extraction, chemical analysis, and pharmacological evaluation. Refractometric analyses were made on BTH and control grapes to evaluate sugar concentration, and a range between 17.5−18.0 °Brix was found in both groups in several replications. The alcohol grade in wine was 13.0%, v/v, and the residual total sugars in wine were undetectable (<0.5 g/L).

Microvinifications were performed in triplicates. Grapes (25 kg) from control and BTH-treated vines were randomly sampled, destemmed, and crushed into stainless steel vats. A typical red-wine-making process was performed on them. At the end of the malolactic fermentation, wines were transferred into bottles. Once bottled, wines were held for 3 months at 10 °C before analysis.

Sample Preparation for Anthocyanin Analysis. Anthocyanin extraction was performed from skins of 20 frozen berries, randomly selected from each sample; berries were peeled without thawing, and the obtained skins were weighed. To standardize the process, 5 g of skin samples was used for each polyphenol extraction. After the addition of 100 mL of 95% methanol (MeOH), the skin samples were kept in the dark at 4 °C overnight; the extraction mixture was filtered, and skins were extracted again with 50 mL of MeOH for 2 h. The combined extracts were dried in a rotary evaporator at 40 °C, and residues were redissolved in 10 mL of MeOH/perchloric acid (0.3%) in water (23:73, v/v). All extracts were kept in foil-wrapped glass vials at −20 °C. The experimental wines were analyzed by direct injection in reverse phase high-performance liquid chromatography (RP-HPLC).

HPLC Analysis of Anthocyanins. Chromatographic separation, for both wines and berry skin extracts, was performed on a Purospher LiChroCART RP-18 HPLC column (4.6 × 250 mm, particle size = 5 μm), protected by a LiChroCART precolumn (4 × 4 mm) (Merck, VWR International). Elutions were carried out using a multilinear gradient of 0.3% perchloric acid in water (solvent A) and absolute methanol (solvent B), at a flow rate of 0.45 mL/min (*9*). The gradient elution profile was as follows: 0 min, 27% B, 73% A; 1−32 min, 43% B, 57% A; 32−45 min, 68.5% B, 31.5% A; 45−47 min, 100% B; 3 min constant 100% B. Samples were centrifuged at 10000*g* for 30 min and then filtered using a polytetrafluoroethylene (PTFE) 0.45-μm membrane syringe filter (Corning, Inc.). The injection volume of each sample was 10 μL, for berry skin extracts, and 25 μL for wines. Fluorometric detections were recorded for 60 min at λ_{ex} = 330 nm and λ_{em} = 374 nm for anthocyanin quantification. A wavelength of 520 nm was used for the absorbance detector in a separate channel.

Anthocyanin concentrations were expressed as milligrams per liter malvidin equivalents (ME), correlated to the calibration curve at the following five concentration points: 0.01, 0.05, 0.1, 0.2, and 1.0 mg/mL of malvidin.

Aortic Ring Preparation and Bioassay of Berry Skin Extracts. White male New Zealand rabbits (Harlan Italy, S. Pietro al Natisone, UD, Italy) (1.8−2.4 kg body weight) were anesthetized with xilazine (5 mg/kg) and ketamine (5 mg/kg). Aortas were gently removed to avoid damage to the endothelial lining, cleared of fat and connective tissue, and cut into 2−3 mm wide rings. Four randomly chosen rings were connected by surgical silk to form a chain and mounted under a resting tension of 20 mN (2 g) in a glass organ bath filled with Tyrode−Ca−HEPES (2.6 mM KCl, 1.05 mM $MgCl_2$, 137 mM NaCl, 12.1 mM $NaHCO_3$, 5.6 mM glucose, 0.9 mM $CaCl_2$, 4.2 mM HEPES, 0.35 mM ascorbic acid, pH 7.4), bubbled with carbogen gas (5.0% CO_2 in O_2) and with a temperature of 37 °C. The choice of this experimental protocol is justified by the fact that during aortic ring isolation and preparation, inadvertent damage to the endothelium may take place, which may cause significant response variability of single rings; by joining several rings together, the risk of such a variability is minimized, and optimal response reproducibility is granted. After an equilibration period of 1 h, under a basal tension of 2 g, changes in isometric contraction induced by noradrenaline (NA) (0.1−3 μM; final tension = 2.6 g) were monitored by a force transducer (model 7004; Basile, Varese, Italy) connected to a pen recorder (Gemini 7070 Basile). Responses to an endothelium-dependent vasodilating agent (3 μM acetylcholine) were tested following enhancement of vascular tone with a submaximal (1 μM) concentration of NA to verify endothelium integrity. Vessels that gave a relaxation of <50% were not used (*10*). Vessel responses were highly reproducible throughout the experiments, the length of which averaged 3.5 h.

Vessel response was expressed as percent of noradrenaline-induced contraction.

Experimental animals were housed and used according to Italian Law n.116, January 27, 1992, recipient of the European Community Directive 86/609/CEE.

Preparation of Human Umbilical Vein Endothelial Cells (HUVECs). HUVECs were isolated as described (*11*), cultured to confluence on collagen-coated dishes (Sigma-Aldrich, Milan, Italy; and Celbio, Milan, Italy) in medium 199 supplemented with 20% fetal bovine serum (Gibco-Life Technologies Milan, Italy), 100 μg/mL endothelial cell growth factor, 100 μg/mL heparin, 100 units/mL penicillin, and 0.1 mg/mL streptomycin (Sigma-Aldrich, Milan, Italy). Cells were kept at 37 °C under 5% CO_2/95% air and used between passages 1 and 2. Cells were grown to confluence in Petri dishes (60 mm) in the presence of 2 mL of medium and treated with Merlot and BTH-Merlot grape skin extracts, MeOH (1:1000), ACH (1 μM), and SNP (1−10 μM) for 5 min. At the end of the exposure, the culture medium was removed, and 600 μL of 0.1 M HCl was added to the cell plates and incubated for 20 min at room temperature. Cells were scraped off the surface with a cell scraper, and the mixture was dissociated by pipetting up and down until the suspension was homogeneous. Cells were then transferred to appropriate tubes and centrifuged at 1000*g* for 10 min. The supernatant was then decanted into a clean test tube, and c-GMP levels were directly assayed according to a previously described enzyme immunoassay method (*12*), using a commercially available kit (SPI-BIO, Saclay, F).

Presentation of Results. The data related to anthocyanin content result from the pooling of three different extracts derived from mature clusters collected in 20−30 parcels, each composed of 4−5 plants, which were selected and assigned to BTH or control treatment according to a randomized block sampling protocol (*13*). Results were analyzed by Duncan's new multiple-range test.

The concentration−response curves of vasodilation were analyzed and drawn by means of the computer program ALLFIT (*14*), and evaluation of the statistical significance of the parameter difference was based on Student's t test for paired data analysis.

A P value of <0.05 ($P < 0.05$) was considered to be statistically significant.

RESULTS

Three main groups of anthocyanins were detected after extraction of berry skin tissues from BTH-treated and control plants, that is, five monoglucosides of the anthocyanidins delphinidin, cyanidin, petunidin, peonidin, and malvidin, as well as the corresponding acetylated and *p*-coumaroyl derivatives.

Table 1. Concentration of Monoglucoside of Anthocyanidins, as Well as the Corresponding Acetylated and *p*-Coumaroyl Derivatives, in Berry Skin Extracts Obtained from Control (CTRL) and BTH-Treated Grapevines[a]

	CTRL (mg/L)	BTH (mg/L)
delphinidin	51.45 ± 0.17	183.7 ± 2.2*
cyanidin	4.9 ± 0.04	15.75 ± 0.15*
petunidin	59.85 ± 0.66	211.0 ± 0.64*
peonidin	33.6 ± 1.1	102.9 ± 0.57*
malvidin	657.6 ± 0.54	1908.9 ± 11.5*
acetylated derivatives	313.9 ± 0.97	1072.0 ± 6.4*
p-coumaroyl derivatives	169.0 ± 1.31	1879.8 ± 17.3*

[a] Data are expressed as mg/L of malvidin equivalents (ME), correlated to the calibration curve at the following concentrations: 10, 50, 100, 200, and 1000 mg/L of malvidin. *, $P \leq 0.01$, by Duncan's test vs CTRL.

Table 2. Concentration of Monoglucoside of Anthocyanidins, as Well as the Corresponding Acetylated and *p*-Coumaroyl Derivatives in Experimental Wines Obtained from Control (CTRL) and BTH-Treated Grapevines[a]

	CTRL (mg/L)	BTH (mg/L)
delphinidin	41.0 ± 0.005	51.1 ± 3.3*
cyanidin	1.8 ± 0.84	1.0 ± 0.4
petunidin	82.3 ± 9.6	96.2 ± 6.4*
peonidin	51.9 ± 2.8	41.1 ± 4.4*
malvidin	919.8 ± 76	1196.0 ± 110*
acetylated derivatives	459.9 ± 68	670.3 ± 130*
p-coumaroyl derivatives	130.2 ± 34	199.6 ± 51

[a] Data are expressed as mg/L of malvidin equivalents (ME), correlated to the calibration curve at the following concentrations: 10, 50, 100, 200, and 1000 mg/L of malvidin. *, $P \leq 0.01$, by Duncan's test vs CTRL.

The whole anthocyanin content in berry skin extracts strongly increased after BTH treatment as shown by RP-HPLC quantitative analysis (**Table 1**). On the other hand, experimental wines, made with BTH-treated and untreated grapes, collected at Conegliano Veneto and analyzed 6 months after alcoholic fermentation, differed from the respective berry extracts with regard to anthocyanin fingerprint, thus showing that these compounds were somewhat affected by fermentation (**Table 2**). However, the reported differences were not due to anthocyanin composition, but rather arose from their amounts. Hence, wine from control grapes contained a higher amount of malvidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and acetylated anthocyanidins than the corresponding berry extracts, whereas, in BTH samples, the amount of the detected compounds as a whole was lower in wine than in berries. Moreover, from a comparison of the wines, malvidin-3-glucoside was the major monoglucoside, although BTH treatment did not raise significantly the whole anthocyanin pool. Particularly, in BTH wine, lower amounts of peonidin-3-glucoside and cyanidin-3-glucoside were identified, whereas coumaric and acetic esters of anthocyanidin augmented.

Control berry skin extracts at different final dilutions caused a concentration-dependent relaxation of noradrenaline (NA)-precontracted aortic rings with an extrapolated EC_{50} observed at the dilution of 1:4400. This effect was completely abolished by mechanical removal of endothelium, as well as by pretreatment with compound 1*H*-(1,2,4)oxadiazolo(4,3-*a*)quinoxalin-1-one (ODQ, 10 μM), a selective inhibitor of NO-sensitive guanylate cyclase activity (*15*) (**Figure 1**). Changes in tension by NA in the normal and ODQ-treated conditions did not differ. Berry skin extracts from preharvest BTH-treated grapevines also caused relaxation of NA-precontracted aortic rings, with a

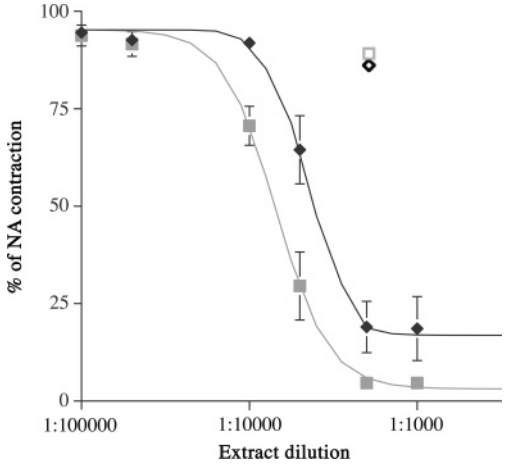

Figure 1. Vasodilation of isolated rabbit aortic rings induced by different dilutions of berry skin extracts obtained from control and BTH-treated grapevines: (◆) Merlot; (gray squares) BTH-Merlot; (◇) Merlot + 10 μM ODQ; (□) BTH-Merlot + 10 μM ODQ. The results represent the average of five different isolated organ preparations using the extracts reported in **Table 1**. Statistical analysis of the two different curves using ALLFIT showed a significant difference ($P < 0.05$) between the EC_{50} of control (1:4400) and that of BTH-treated grapevine (1:8000).

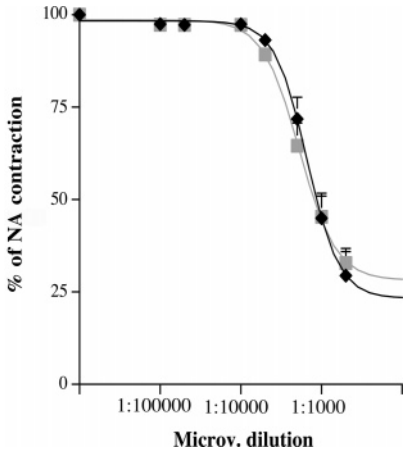

Figure 2. Vasodilation of isolated rabbit aortic rings induced by different dilutions of experimental wine obtained from control and BTH-treated grapevines: (◆) Merlot; (gray squares) BTH-Merlot. The results represent the average of five different isolated organ preparations using the experimental wines reported in **Table 2**.

concentration response curve that was shifted to the left of the control curve in a parallel fashion and with an extrapolated EC_{50} observed at the final dilution of 1:8000 ($P < 0.05$ vs control, paired test); this effect was also abolished by vessel treatment with ODQ. Ethanol at the concentration present in tested extracts did not affect vascular tone. Different aliquots of control microvinificates caused a concentration-dependent, NO-dependent vascular relaxation, with a potency and efficacy that were indistinguishable from that recorded using similar aliquots of BTH microvinificates (**Figure 2**). This means that the enhancement of the vasorelaxation effect produced by BTH plant treatment on the berry skin extracts is not retained in the wine therefrom.

In selected experiments 1:1000 dilutions of control and BTH-treated plant extracts (a dilution that granted complete vascular relaxation) were tested separately for their ability to stimulate soluble guanylate-cyclase enzyme activity in HUVECs.

Control berry skin extracts increased cGMP formation (1.54-fold increase, $n = 6$) when compared to untreated cells as well

Table 3. Formation of Cyclic 3′,5′-Guanosine Monophosphate (cGMP) in Human Umbilical Vein Endothelial Cells Induced by Berry Skin Extracts from Untreated (CTRL) and BTH-Treated Grapevines[a] (Cv. Merlot)

	c-GMP (pmol/μg of proteins)
CTRL	3.33 ± 2.14
MeOH (1:1000)	3.59 ± 1.85
Merlot (1:1000)	5.13 ± 4.19*
BTH-Merlot (1:1000)	9.49 ± 7.14*
1 μM Ach	4.36 ± 1.8*
1 μM SNP	3.79 ± 0.83
10 μM SNP	15.11 ± 1.89*

[a] Acetylcholine (Ach) and sodium nitroprusside (SNP) were used as reference controls. *, $P \leq 0.05$, by Duncan's test vs CTRL.

as cells treated with vehicle (MeOH); extracts from preharvest BTH-treated grapevines significantly stimulated ($P < 0.01$, $n = 6$) enzyme activity over blank values (2.85-fold increase vs control). Sodium nitroprusside (SNP, 1 μM), a nitrovasodilator that acts by releasing NO, did not alter basal guanylate cyclase activity, whereas 10 μM SNP markedly stimulated cGMP formation in HUVECs (4.54-fold over blank). Acetylcholine (Ach, 1 μM) caused a moderate but statistically significant increase in cGMP formation (**Table 3**).

DISCUSSION

There is well-documented epidemiological evidence that red wine can be beneficial in reducing the risk of cardiovascular disease, beyond that expected from its alcohol content. Red wine contains many compounds that might influence cardioprotection: in particular, polyphenols may trigger NO-dependent cell signaling, including endothelial-dependent vasorelaxation, inhibition of platelet aggregation, modulation of primary hemostasis, and prevention of experimental thrombosis in rats (*5*). In addition, red wine increases the expression of human endothelial nitric oxide synthase (*4*), a mechanism that may contribute further to its beneficial cardiovascular effects. Altogether, the characteristics of the cardioprotection associated with wine consumption are indicative of mechanisms pivoting on increased NO bioavailability and have led to the provocative hypothesis that the NO pathway may represent the molecular target of polyphenol-dependent cardioprotection (*16*).

Plants produce a great variety of secondary metabolites having importance linked to defense traits against biotic and abiotic stresses. With regard to resistance against pathogens, these compounds can be classified as inducible phytoalexins, the concentrations of which in plant tissues increase strongly in response to microbial challenge or chemical treatment (*6*, *7*). Among polyphenols, anthocyanins are prominent phytoalexins present in grape skin, and their biosynthesis during the ripening phase causes their accumulation in the berry skin. Several agroecological factors, including climate, irrigation, and pathogen infections, have been related to their accumulation in grape (*17*, *18*).

Plant resistance activators are a class of natural or synthetic compounds that stimulate plant defense mechanisms (*3*); among them one of the most interesting is benzothiadiazole (BTH), which induces systemic acquired resistance (SAR) of the plant against different pathogens. The only side effect of chemically induced resistance, that is, fitness costs, depends on the plant system, occurring preferentially in certain crops, but not in others (*19*, *20*). During this investigation we did not detect any adverse effect in viticultural parameters of BTH-treated grapevine in terms of growth, yield per vine, or berry size.

We reasoned that BTH treatment of grapevine at preharvest could promote the synthesis of secondary metabolites, such as anthocyanins, probably due to BTH enhancement of the activity and/or transcription of phenylalanine ammonia-lyase (PAL), the key enzyme of the phenylpropanoid pathway, as reported in an array of pathosystems (reviewed in ref *8*), and thereby fostering their nutraceutical−cardioprotective potential linked to the NO system. Furthermore, in a previous work (*6*), we postulated that BTH could induce the enhancement of chalcone synthase, at the major branching of polyphenol/anthocyanin biosynthesis. Indeed, the results reported here clearly indicate that in postharvest berries, BTH triggers a significant increase of anthocyanin content, including five monoglucosides of the anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin) as well as the corresponding acetylated and *p*-coumaroyl derivatives. This is in line with previously reported data (*6*), where BTH improved the synthesis of grapevine phytoalexin resveratrol, thus inducing resistance to *Botrytis cinerea*. However, the experimental wines made from BTH-treated grapes showed a moderate change of anthocyanin concentrations when compared to wines made from control grapes.

During the first year of wine maturation, when it is in bulk storage before bottling and in an aging phase, new pigments arise following copigmentation phenomena, such as anthocyanin condensation with flavanols, other anthocyanins, proanthocyanidins, and piruvic acid. Evidence of condensation occurrence is represented by the disappearance of monomeric anthocyanins, progressively replaced by polymeric derivatives, and, ultimately, wine color stabilization occurs. In view of this, the anthocyanin pattern of BTH-wine is significantly different from that of the grapes used for making it. A possible explanation could be that the higher pool of pigments, reported in BTH-berry skin extracts, functions as a source of compounds differently partitioned by two different processes: monomeric and polymeric anthocyanin biosynthesis. However, we did not carry out the analysis of polymeric derivatives, and, thus, we can merely speculate on a possible unbalanced allocation of free anthocyanin reservoir toward two different sinks. Anyway, the anthocyanin content of BTH-wine was almost invariably higher than that of the control one, despite the difference between wine and berry skin extracts from treated grapes.

Such an increase in pharmaconutrient content of BTH-treated berry skins is closely accompanied by a significant improvement of the NO-releasing capacity of berry skin extracts, as shown by the parallel leftward shift of their concentration−response curve on precontracted aortic rings. The vascular relaxation induced by berry skin extracts fully complied with the NO paradigm, in that it was abolished by endothelium removal and by pretreatment of aortic specimens with a guanylate cyclase inhibitor compound, ODQ. Moreover, the capacity of berry skin extracts to stimulate guanylate-cyclase enzyme activity in HUVECs provides further indirect evidence of NO involvement, because this enzyme represents its privileged intracellular target. The berry skin extracts that we have examined may undoubtedly contain a wide variety of compounds of different chemical structures. In this regard, we recently reported melatonin in grapevine (cv. Merlot), as well as its augmentation due to elicitation (*21*). Anyway, it is beyond the purpose of this work to ascribe any single pharmacological effect (in this case the vasorelaxant effect) to any single compound. Therefore, the reported effect could result from interactions between several components, possibly belonging to different structural subclasses (*22*) and may be described as general, but it is tempting to

propose that the active agent is represented by anthocyanins. Indeed, the role of the anthocyanin fraction of red wine on endothelial NO generation has already been investigated. In rat thoracic aorta, anthocyanins induced a higher endothelium-dependent vasorelaxation than other fractions (*23*). In bovine aortic endothelial cells anthocyanins elicited cytosolic free calcium release from intracellular stores, which, in turn, stimulated NO production (*24*). Thus, our hypothesis on the putative anthocyanin activity is in agreement with previously reported data, although obtained in different experimental models. However, further investigations are now in progress to characterize the class of compounds involved in the pharmacological effect. The NO-dependent vasodilation of berry skin extracts here reported clearly represents an effect observed at pharmacological concentrations, and any extrapolation to an actual nutraceutical value will require further in vivo studies.

The change of anthocyanin content reported in BTH wine versus BTH berry extracts may be due to their copolymerization mainly with oligomeric and polymeric proanthocyanidins. In a previous work carried out using the same Merlot cultivar as the one reported in this investigation, we showed that BTH treatment greatly enhanced oligomeric and polymeric proanthocyanidin synthesis in berries (*7*). Thus copolymerization, a process that takes place during the malolactic fermentation of wine, may subtract a share of anthocyanin content, thus explaining the loss of anthocyanins observed, as well as the reduced vascular relaxation induced by BTH wine versus BTH berry extracts. However, copolymerization may be of interest from an enological point of view, because wine color stabilization is markedly improved.

The toxicological risk associated with BTH, as well as its acid derivative [*S*-methyl benzo-(1,2,3)-thiadiazole-7-carboxylic acid, CGA 210-007], is very low (*25*); both compounds, when used for plant treatment at concentrations similar to those used in our study, are completely degraded and are commonly used for their ability to protect, for example, tomato plants against bacterial diseases (*26*). Moreover, as recognized by the European Commission (Directorate E-Food safety: plant health, animal health and welfare, May 2002), BTH and its derivative fulfill the EEC safety requirements for plant protection products and have no harmful effects on human or animal health.

In conclusion, we propose that plant (*Vitis vinifera*) treatment with the resistance activator BTH at preharvest may represent an interesting strategy to enrich the nutraceutical potential of grape.

ABBREVIATIONS USED

NA, noradrenaline; NO, nitric oxide; SNP, sodium nitroprusside; BTH, *S*-methyl benzo-(1,2,3)-thiadiazole-7-carbothioate; ODQ, 1*H*-(1,2,4)-oxadiazolo(4,3-*a*) quinoxalin-1-one.

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