

Antioxidant and Cellular Activities of Anthocyanins and Their Corresponding Vitisins A—Studies in Platelets, Monocytes, and Human Endothelial Cells

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During red wine aging, there is a loss of anthocyanins and the formation of various other pigments, so-called vitisins A, which are formed through the chemical interaction of the original anthocyanins with pyruvic acid. The objective of this study was to investigate the antioxidant activities of the most abundant anthocyanins present in red wine (glycosides of delphinidin, petunidin, and malvidin) and their corresponding vitisins A. Anthocyanins exhibited a higher iron reducing as well as 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonate) and peroxy radical scavenging activity than their corresponding vitisins A. Delphinidin showed the highest antioxidant effect of the tested compounds in all of the assays used. Furthermore, we studied the effect of anthocyanins and vitisins A on platelet aggregation and monocyte and endothelial function. Anthocyanins and vitisins did not affect nitric oxide production and tumor necrosis factor- α (TNF- α) secretion in lipopolysaccharide plus interferon- γ -activated macrophages. Furthermore, anthocyanins and vitisins did not change collagen-induced platelet aggregation in vitro. However, anthocyanins and to a lesser extent vitisins exhibited protective effects against TNF- α -induced monocyte chemoattractant protein production in primary human endothelial cells.

KEYWORDS: Anthocyanin; vitisin A; antioxidant activity; nitric oxide production; TNF- α secretion; MCP-1 production; platelet aggregation

INTRODUCTION

Anthocyanins are a group of phenolic compounds responsible for the red–blue color of many fruits and vegetables. They are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium or flavilium salts (1). The main dietary sources of anthocyanins include red-colored fruits and vegetables and red wine. The intake of anthocyanins in humans has been estimated to be 180–215 mg/day (2) in the United States, which is considerably higher than the intake of other flavonoids (23 mg/day) including quercetin, kaempferol, myricetin, apigenin, and luteolin (3). Epidemiological studies suggest that a moderate consumption of anthocyanins may be, at least partly, associated with a protection against coronary heart disease (4, 5). During red wine aging, there is a loss of anthocyanins and it appears that other pigments, so-called vitisins A (Figure 1), are formed

through the interaction of the original anthocyanins with pyruvic acid (6). The structure of vitisin A is based on the anthocyanidin-3-glucoside with an additional C₃O₂ between position C4 and the 5-hydroxyl group of the molecule (7). The chemical interaction between anthocyanins and pyruvic acid has been shown to influence the color of red wine and may also impact the antioxidant and biological properties of anthocyanins.

Although there is evidence that anthocyanins provide beneficial effects to human health, their cellular mechanism of action remains largely unknown. The purpose of the present study was 2-fold: to analyze the antioxidant activity of the most abundant anthocyanins present in red wine, Dp-3-glu, Pet-3-glu, and Mv-3-glu, in comparison to their corresponding vitisins A and to study the effect of anthocyanins and vitisins on NO production and TNF- α secretion in macrophages, collagen-induced platelet aggregation, and MCP-1 production in primary HUVEC. We have chosen these three cell types since they play a key role in the pathogenesis of atherosclerosis.

MATERIALS AND METHODS

Isolation and Purification of Anthocyanins. Anthocyanins (Dp-3-glu, Pet-3-glu, and Mv-3-glu) were isolated from a methanol–acid red grape skin extract by semipreparative high-performance liquid

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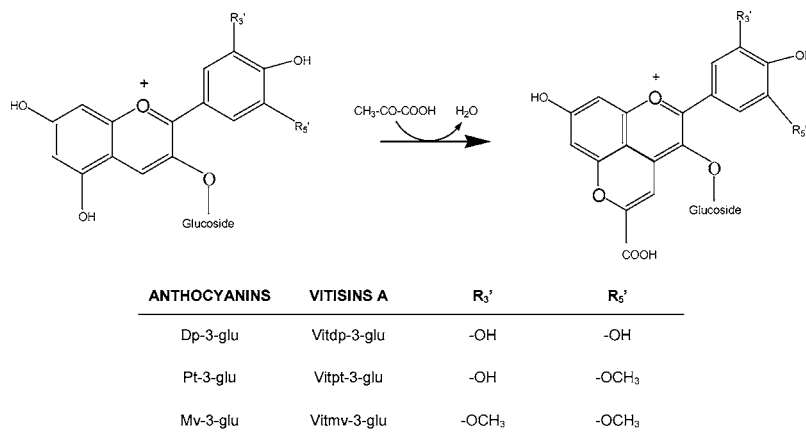


Figure 1. Reaction of anthocyanins (Dp-3-glu, Pt-3-glu, and Mv-3-glu) and pyruvic acid to form vitisins A (Vitdp-3-glu, Vitpt-3-glu, and Vitmv-3-glu), which occur during red wine aging.

chromatography (HPLC) using a Waters 600 chromatograph. The column was an Ultracarb ODS, 5 μ m, 250 mm \times 10.00 mm (Phenomenex). The solvents were 5% acetic acid (A) and methanol (B) with the following gradient: from 10 to 15% B over 15 min, isocratic 15% B for 5 min, from 15 to 20% B over 10 min, from 20 to 25% B over 10 min, from 25 to 30% B over 10 min, from 30 to 45% B over 10 min, and from 45 to 10% B over 10 min at a flow rate of 3 mL/min. Detection was carried out at 520 nm.

Vitisin A Synthesis. The synthesis of vitisins A was performed as described previously by Romero et al. (7). Pyruvic acid was added to pure anthocyanin and dissolved in potassium hydrogen tartrate buffer containing 10% ethanol, in a molar ratio of pyruvic acid to pure anthocyanins of 300:1. The pH was adjusted to 3.7 by the addition of Na₂CO₃, and the solution was incubated at 32 °C in the dark in the presence of air. The identity of the vitisins A was confirmed in the liquid chromatography–mass spectrometry (LC-MS) analysis, and the kinetics of formation was followed by HPLC analysis as described below.

The purity of anthocyanins and vitisins A was tested by HPLC analysis following the method of de Pascual-Teresa et al. (8) with slight modifications. Briefly, Hewlett-Packard 1100 HPLC equipment was used with a quaternary pump and a photodiode array detector. The column was an Aqua C18, 5 μ m (150 mm \times 4.6 mm) (Phenomenex), thermostated at 35 °C. Solvents were 0.1% trifluoroacetic acid (A) and acetonitrile (B) with the following gradient: isocratic 10% B for 5 min, from 10 to 15% B over 15 min, isocratic 15% B for 5 min, from 15 to 18% B over 5 min, and from 18 to 35% B over 20 min at a flow rate of 0.5 mL/min. The detection was made in the photodiode apparatus, selecting 520 nm as the preferred wavelength.

The identity of the vitisins A synthesized was confirmed by HPLC using a dual on-line detection by diode array spectrophotometry and MS. MS was performed using a Finnigan LCQ equipped with an API surface, using an electrospray ionization interface. The HPLC system was connected to the probe of the mass spectrometer via the diode array detector cell outlet, using polyethyl etherketone tubing. Both the auxiliary and the sheath gas were a mixture of nitrogen and helium at flow rates of 1.2 and 6 L/min, respectively. The capillary temperature was 195 °C, and the capillary voltage was 4 V. The MS detector was programmed to perform a series of three consecutive scans: a full scan from 120 to 1500 amu, an MS² scan of the most abundant ion in the full mass, and an MS³ scan of the most abundant ion in the MS². The normalized energy of collision was 45%. The spectra were recorded in the positive ion mode.

Antioxidant Activity. FRAP. The FRAP assay was performed as previously described (9). The FRAP reagent was freshly prepared each day by mixing together 10 mM TPTZ and 20 mM iron(III) chloride in 0.25 M acetate buffer, pH 3.6. A 100 μ L amount of test components (100 μ M) was added to 3 mL of FRAP reagent, and the absorbance was read at 593 nm (Perkin-Elmer UV/Vis Lambda Bio 20) after incubation at room temperature for 6 min, using the FRAP reagent

with distilled water as a blank. Data were expressed relative to values obtained for ascorbic acid (200 μ M) and expressed as ascorbic acid equivalents.

TEAC. Sixteen hours prior to the assay being performed, the ABTS radical was prepared by adding 5 mL of a 4.9 mM potassium persulfate solution to 5 mL of a 14 mM ABTS solution (10). This solution was diluted in distilled water to yield an absorbance of 0.70 at 734 nm (Perkin-Elmer UV/Vis Lambda Bio 20). The final reaction mixture contained 10 μ L of standard or test compound in 1 mL of ABTS solution. The samples were vortexed for 10 s, and 6 min after the addition, the absorbance was recorded and compared to the ABTS radical solution plus distilled water. A standard curve was obtained by using Trolox as an internal standard (range, 0–100 μ M). Different dilutions of the test components were assayed in order to express the TEAC values as the μ mol of Trolox with the antioxidant capacity corresponding to 1.0 μ mol of the test substance (11).

ORAC. The procedure was based on a previous report of Cao et al. (12). In the final assay mixture (3.35 mL total volume), sodium fluorescein (7.0×10^{-8} M) was used as a target of free radical damage, AAPH (17 mM) was used as a peroxy radical generator, and the Trolox was used as a control antioxidant standard. The test components were added to the assay mixture at 5, 10, and 15 μ M in every case. The fluorescent filters were set to pass the light with an excitation wavelength of 493 nm and an emission wavelength of 513 nm, and the fluorescence was measured every 5 min after the addition of AAPH. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescein decay curve and were expressed as Trolox equivalents. The relative ORAC values (Trolox equivalents) were calculated as relative ORAC value = [(AUC_{sample} - AUC_{Blank})/(AUC_{Trolox} - AUC_{Blank}) \times (molarity of Trolox/molarity of sample)].

Macrophages. RAW 264.7 cells, a murine cell line of monocyte macrophages (obtained from the European Collection of Cell Culture, Salisbury, U.K.) were maintained at 37 °C in 5% CO₂ according to standard protocols (13). The medium consisted of Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μ g/mL streptomycin (Sigma, Poole, U.K.). The cells were plated in six well plates at a density of 5×10^5 in 3 mL of medium, cultured for 48 h until the cells reached 80% confluence, and then treated as follows. After pretreatment with anthocyanins, vitisins A, both up to 50 μ M, or DMSO for 6 h, cells were stimulated with 500 ng/mL LPS plus 100 U/mL IFN- γ (Sigma) for 24 h.

Endothelial Cells. Primary HUVEC (Biowhittaker, Wokingham, U.K.) were grown in cell basal medium (HEPES and bicarbonate-buffered medium), which was supplemented with cell growth supplement (containing heparin, hydrocortisone, human epidermal growth factor, and fetal bovine serum) and antibiotics (gentamicin and amphotericin B) (Large Vessel Endothelial Cell Growth Medium Package, TCS Cell Works Ltd., Liverpool, U.K.). Passages were performed according to standardized protocols using trypsin/ethylene-

diaminetetraacetic acid, buffered saline rising solution, and trypsin blocking solution (Passage Pack—ZHR-9941, TCS Cell Works Ltd.) and by diluting the cells 1:4. HUVEC cells, within passages 2–4, were seeded in 3 mL of medium in six well plates and cultured until they reached 80–90% apparent confluence. After pretreatment with 50 μ M anthocyanins, vitisins A, or DMSO for 24 h, cells were stimulated with 40 ng/mL of TNF- α (Sigma) for 24 h.

Cell Viability. The uptake of neutral red dye was used to measure cell viability as described previously (14). Macrophages and HUVEC were pretreated with anthocyanins and vitisins A for 6 or 24 h, respectively. After activation of RAW264.7 macrophages with LPS plus INF- γ and HUVEC with TNF- α for 24 h, the culture medium was removed and replaced with fresh medium containing 60 μ g/mL of neutral red for 3 h at 37 °C. Following incubation with the neutral red dye, the medium was removed and the cells were extracted using a solution comprising 50:49:1 (v/v/v) ethanol, water, and glacial acetic acid. The absorbance was recorded at 540 nm using a microplate reader. Neither anthocyanins nor vitisins A exhibited any cytotoxic effect in macrophages and HUVEC up to a concentration of 100 μ M.

NO Production. The NO production was assessed by measurement of nitrite concentration (NO₂⁻) in the medium using the Griess reaction. Supernatants of cultured macrophages were collected and deproteinized with 0.3 M NaOH and 0.3 M ZnSO₄. An equal volume of the Griess reagent (1% sulfanilamide/0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride/2.5% H₃PO₄) and the deproteinized samples was incubated for 10 min at room temperature protected from light. The nitrite concentration was determined by measuring the absorbance at 548 nm against a standard curve for sodium nitrite (15).

TNF- α Secretion in RAW 264.7. Supernatants collected for NO production assessment were also used to determine TNF- α secretion in RAW 264.7. Upon collection, samples were centrifuged at 13 000 rpm for 10 min and the supernatants were kept at -80 °C until they were analyzed. The TNF- α secretion was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantine M mouse TNF- α immunoassay, R&D Systems Europe, Abingdon, U.K.).

MCP-1 Secretion in HUVEC. Upon collection of the supernatant from HUVEC cells, samples were centrifuged at 13 000 rpm for 10 min and the supernatants were kept at -80 °C until analyzed. The secretion of MCP-1 by HUVEC was measured by using a commercially available ELISA kit (R&D Systems Europe).

Platelet Isolation and Platelet Aggregation Measurement. Venous blood samples were obtained from volunteers within the Hugh Sinclair Unit of Human Nutrition at the University of Reading. Blood for platelet aggregation studies was drawn into 4.5 mL sodium citrate vacutainer tubes (Becton Dickinson, Plymouth, U.K.) and kept at room temperature until analysis. Platelet aggregation studies were performed in a two channel whole blood impedance aggregometer (model 590, Chronolog Corporation, Labmedics Ltd., Cheshire, U.K.). Whole blood was diluted (500 μ L), 1:1, with PBS, placed in cuvettes with a stir bar, and warmed to 37 °C. Two microliters of the anthocyanins, vitisins A, or DMSO controls was then added, and the sample was stirred for 10 s. The blood samples were incubated with the test compound for 10 min. The aggregometer's impedance probe was placed into the warmed blood, and the baseline impedance was set to zero on the chart recorders. Collagen (1.5 μ g/mL) was added to the cuvette at time zero, and the impedance increased proportionally to the amount of platelet aggregation on the impedance probe. The maximum aggregation was measured at 6 min with the extent of aggregation expressed in ohms of the impedance using Aggrolink software (Chronolog Corporation, Labmedics Ltd.). Control aggregation curves were obtained in the absence of anthocyanins and vitisins A and were compared with the curves obtained in the presence of the test compound. The results were used to calculate the percentage inhibition (100 - percentage of aggregation). Genistein (100 μ M) was used as a positive control for this assay.

Statistical Analysis. Data are presented as means \pm standard deviations (STDEV) of between three and five independent experiments performed in duplicate. Statistical analysis was carried out using analysis of variance and Student's *t*-test (SPSS for Windows version 10.0).

RESULTS

Test Compounds. Purities of the all anthocyanins and vitisins A used in this work, which were tested by HPLC analysis, were 93–94%. The identities of the vitisins A synthesized, Vitdp-3-glu, Vitpt-3-glu, and Vitmv-3-glu, were confirmed by LC-MS, which showed (Figure 2), for their peaks, molecular ions at *m/z* 533, 547, and 561, respectively. In their MS-MS spectra, major fragments appeared at *m/z* 371, 385, and 399, respectively (-162 amu, loss of a glucose moiety), corresponding to the aglycons of the corresponding glycosides of vitisins A.

For all experiments, the anthocyanins as well as the vitisins A were dissolved in DMSO and stored at -80 °C. When test compounds in DMSO were added to the medium, the final DMSO concentration was \leq 0.1% (v/v), and in every case, a control containing the same amount of DMSO was done.

FRAP, TEAC, and ORAC Values. FRAP values indicate the ability of anthocyanins and vitisins A to reduce Fe³⁺ to Fe²⁺. As summarized in Figure 3A, both anthocyanins and vitisins A showed a higher iron reducing activity than the reference ascorbic acid. Among the anthocyanins tested, dp-3-glu was the most potent test component, followed by pt-3-glu and mv-3-glu. Vitmv-3-glu showed a slightly higher, although not statistically or significantly different, FRAP value than Vitdp-3-glu and Vitpt-3-glu.

The TEAC assay measures the ability of an antioxidant to scavenge the ABTS free radical. The results are compared to the ABTS scavenging activity of Trolox, giving a Trolox equivalent value. In the TEAC assay, all test compounds showed a higher antioxidant activity than the standard Trolox (Figure 3B). Dp-3-glu was the most potent scavenger of the free ABTS radical (four times greater than that of Trolox), followed by mv-3-glu with a TEAC value of 3.7. The other test compounds exhibited an about 3-fold higher ABTS scavenging activity than Trolox.

The ORAC assay determines the potency of antioxidant to scavenge peroxy free radicals. The anthocyanins as well as Vitdp-3-glu were about 5-fold more potent, and Vitpt-3-glu and Vitmv-3-glu were about 2-fold more potent than Trolox in scavenging the peroxy free radical (Figure 3C).

NO Production and TNF- α Secretion in Monocytes. Following 24 h of activation of RAW264.7 macrophages with INF- γ plus LPS, levels of nitrite and TNF- α increased from basal levels to 80 μ M/10⁶ cells and 2.7 ng/mL, respectively. Pretreatment of RAW 264.7 with anthocyanins and vitisins A did not affect nitrite production or TNF- α secretion. In contrast, 50 μ M genistein was found to reduce TNF- α and NO production by 55 and 49%, respectively, comparable to previously published data (16).

MCP-1 Secretion in Endothelial Cells. A statistically significant inhibitory effect on MCP-1 secretion was found when HUVEC were pretreated with anthocyanins; this inhibition was not evident to the same extent in the case of the corresponding vitisins A (Figure 4). The anthocyanin pt-3-glu (~70% inhibition) was the most potent inhibitor of MCP-1 secretion followed by dp-3-glu (~50% inhibition) and mv-3-glu (~40%). Pretreatment of HUVEC with Vitdp-3-glu inhibited MCP-1 production by 35%, whereas Vitpt-3-glu and Vitmv-3-glu did not affect endothelial MCP-1 secretion.

Platelet Aggregation. Neither anthocyanins nor vitisins A inhibited collagen-induced platelet aggregation at concentrations up to 50 μ M. However, a significant inhibitory effect was evident for genistein (29%), used as a positive control (16).

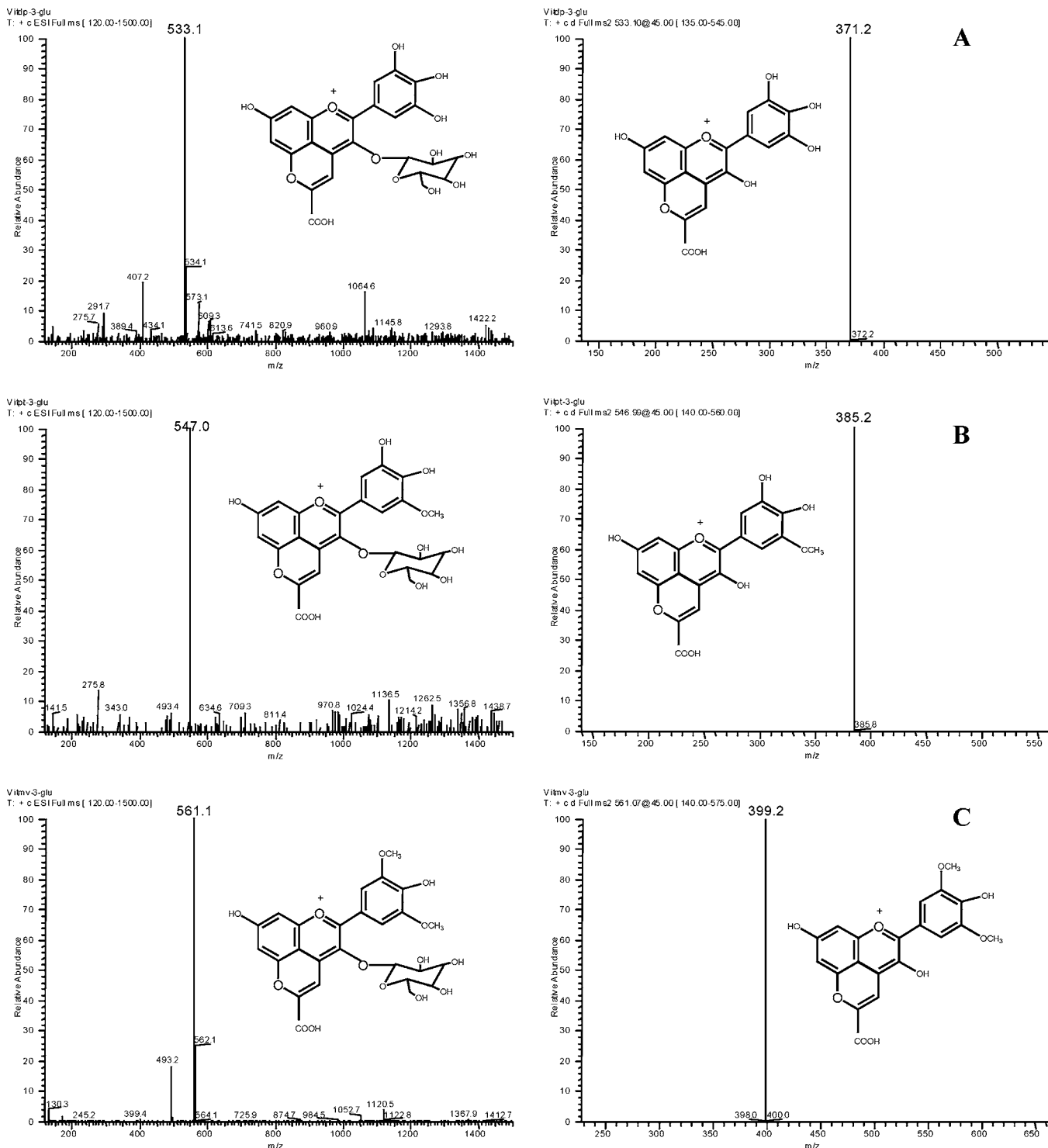


Figure 2. MS and MS/MS spectra of Vitdp-3-glu (A), Vitpt-3-glu (B), and Vitmv-3-glu (C).

DISCUSSION

Anthocyanins are thought to play a role in the prevention of coronary heart disease, but the mechanisms by which they and their corresponding vitisins A mediate these effects have not been fully established. Antioxidant properties are an obvious candidate. However, although antioxidant properties of anthocyanins have been described, the free radical scavenging and iron chelating activities of vitisins A in comparison to anthocyanins are largely unknown. Furthermore, little is known about the cellular effects of anthocyanins and vitisins A in platelets,

monocytes, and endothelial cells. Here, we investigate both the antioxidant and the cellular effects of anthocyanins and vitisins A.

Antioxidant Properties of Anthocyanins and Vitisins A. FRAP, TEAC, and ORAC data indicate that both anthocyanins and vitisins A have the potency to reduce Fe^{3+} to Fe^{2+} and to scavenge the ABTS and peroxy free radicals. The FRAP and TEAC values of anthocyanins decreased in the order dp-3-glu > mv-3-glu = pt-3-glu. The structural difference among the anthocyanins tested is the substitution in the 3'- and 5'-position in the B ring. Dp-3-glu is the only compound studied that

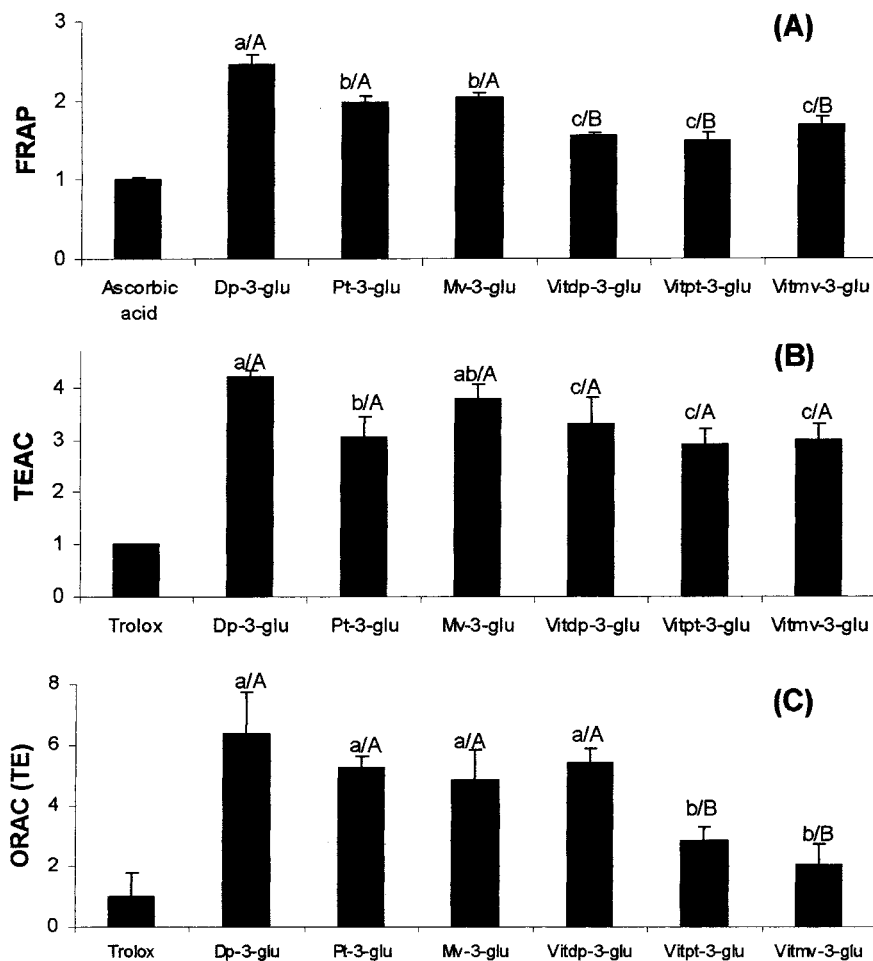


Figure 3. FRAP (A), TEAC (B), and ORAC (C) values for the anthocyanins and vitisins A. Data are the means \pm STDEV of the three independent experiments performed in triplicate. Different lower case letters indicate significant ($P < 0.05$) differences between anthocyanins or vitisins A. Different upper case letters indicate significant ($P < 0.05$) differences between each anthocyanin and its corresponding vitisin A.

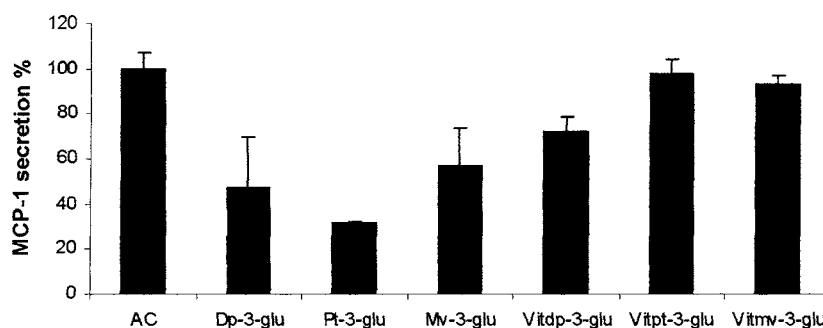


Figure 4. Effect of 50 μ M anthocyanins and vitisins A on MCP-1 secretion in TNF- α -activated HUVEC. Results were expressed as a percentage of MCP-1 secretion. Data were compared to activated controls not treated with anthocyanins or vitisins A.

contains the 3'-, 4'-, and 5'-hydroxy group (gallo catechol structure) in the B ring. Mv-3-glu and pt-3-glu with one and two hydroxyl groups, respectively, in the B ring exhibited a lower antioxidant activity than dp-3-glu indicating that the gallo catechol group is an important determinant for the radical scavenging potential of polyphenols. Furthermore, our data demonstrate that anthocyanins exhibit a higher antioxidant activity than their corresponding vitisins A. Thus, the incorporation of an additional C₃O₂ group in positions 4 and 5 of the molecule decreases the ability of vitisins A to scavenge ABTS and peroxy free radicals. This decrease in antioxidant activity may be due to the lack of the 5-hydroxyl group in the vitisin A molecule, which together with the 7-hydroxyl group contributes to the antioxidant activity of anthocyanins (17).

Cellular Activity of Anthocyanins and Vitisins A. Platelets, macrophages, and endothelial cells were chosen as model systems since these cell types play a critical role in the pathogenesis of thrombosis and atherosclerosis. Endothelial cells appear to be involved from the earliest stages. At sites of endothelial injury, perhaps induced by initial accumulation and modification of lipids, these cells produce chemokines such as MCP-1, which attract monocytes. Subsequently, the monocytes are activated to produce proinflammatory molecules such as TNF- α and NO and accumulate lipids to become the foam cells characteristic of fatty streaks. Platelets may also be involved in these early stages of atherosclerosis, in response to the endothelial damage, and are clearly important in the aetiology of thrombogenesis (18).

Monocyte Function. Monocytes are the principal mediators in inflammation and are involved in the development of atheromatous plaques. Macrophages themselves, once activated, secrete a variety of biologically active molecules, including NO and TNF- α (19).

NO produced by macrophages is a cytotoxic mediator and contributes to the events initiated during host defense (20). It has also been shown that the production of TNF- α is crucial for the synergistic induction of NO synthesis in IFN- γ - and/or LPS-stimulated macrophages (15).

However, overproduction of NO has been associated with oxidative stress and with the pathophysiology of numerous diseases such as septic shock, chronic inflammation, and atherosclerosis (21). In the present study, both anthocyanins and vitisins A did not inhibit NO production or TNF- α in activated macrophages. In contrast to our findings, Wang et al. (22) and Hu et al. (23) reported an inhibitory effect of anthocyanins on LPS-induced NO production in macrophages. However, the coinubation of LPS and anthocyanins may have given rise to this effect since polyphenols suppress LPS activity due to a chemical interaction (24); inhibition of NO production might have been caused by a direct interaction of anthocyanins with LPS, rather than a direct effect on the cell. In the present investigation, macrophages were preincubated with anthocyanins for 24 h and were then washed twice with PBS before the addition of IFN- γ and LPS in order to avoid any direct chemical interaction.

Endothelium Function and Platelet Aggregation. The MCP-1 is known to mediate the recruitment of macrophages to sites of infection or inflammation. A direct role of MCP-1 on atherogenesis has been established. MCP-1 mRNA has been detected in atherosclerotic lesions by in situ hybridization (25, 26). Furthermore, a decrease in atherosclerotic lesion size is observed in mice deficient of the MCP-1 receptor CCR-2, and fewer macrophages and monocytes are present in their aortas (27).

In the present study, the pretreatment of primary human endothelial cells with anthocyanins substantially decreased MCP-1 secretion. Our results are in accordance with data by Youdim and co-workers (28), who found that anthocyanin extracts obtained from blueberries and cranberries were protective against TNF- α -induced MCP-1 expression in human microvascular endothelial cells. It is known that the expression of MCP-1 is regulated at the transcriptional level. MCP-1 transcription is mediated by several transcription factors among which are activator protein-1 and NF- κ B (29). Therefore, it is hypothesized that anthocyanins may regulate TNF- α -induced MCP-1 expression through these transcription factors. Interestingly, Atalay et al. (30) demonstrated that the pretreatment of endothelial cells with two different extracts of berry, containing high amounts of anthocyanins, significantly decreased the NF- κ B DNA binding activity.

The fact that anthocyanins are more potent than vitisins A in inhibiting TNF- α -induced MCP-1 expression may be, at least partly, related to their higher antioxidant potential. Furthermore, stereochemical differences and potential differences in the cellular uptake between anthocyanins and vitisins A should also be taken into account when comparing their biological and cellular properties.

Although there are different molecular mechanisms by which antioxidants may affect platelet aggregation, neither anthocyanins nor vitisins A showed an ability to inhibit collagen-induced platelet aggregation in vitro. However, Ghiselli and co-workers (31) found that the aqueous residue (containing the anthocyanic

fraction) from red wine suppressed ADP-induced platelet aggregation. It should be taken into account that aqueous extracts from red wine contain, beside anthocyanins, other water soluble compounds, which might be responsible for the inhibitory effect on platelet aggregation.

Current data indicate that the chemical interaction between anthocyanins and pyruvic acid as it occurs during red wine aging slightly decreases the antioxidant activity of anthocyanins. Furthermore, our findings indicate that anthocyanins, and to a much lesser extent vitisins A, have protective effects against TNF- α -induced MCP-1 secretion in primary human endothelial cells. Interestingly, neither anthocyanins nor vitisins A did affect monocyte function and platelet aggregation whereas the endothelium was highly sensitive to changes in anthocyanin concentration. It should be noted that the concentrations of anthocyanins used in the present study are higher than their circulating plasma levels in humans. However, cellular exposure to the test components in the present study was acute, i.e., 24 h. Therefore, it would be interesting to assess the effects of long-term exposure of lower, physiologically achievable concentrations of anthocyanins and vitisins A and their metabolites on endothelial cell function.

ABBREVIATIONS USED

ABTS, 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonate); TNF- α , tumor necrosis factor- α ; MCP-1, monocyte chemoattractant protein; NO, nitric oxide; HUVEC, human umbilical vein endothelial cells; Dp-3-glu, delphinidin-3-glucoside; Pet-3-glu, petunidin-3-glucoside; Mv-3-glu, malvidin-3-glucoside; Vitdp-3-glu, vitisin A of delphinidin-3-glucoside; Vitpt-3-glu, vitisin A of petunidin-3-glucoside; Vitmv-3-glu, vitisin A of malvidin-3-glucoside; DMSO, dimethyl sulfoxide; FRAP, ferric reducing ability of plasma; TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; TPTZ, 2,4,6-tripridyl-s-triazine; AAPH, 2,2'-azobis(2-amidinopropane)-dihydrochloride; LPS, lipopolysaccharide; IFN- γ , interferon- γ ; PBS, phosphate-buffered saline; NF- κ B, nuclear factor κ B; ADP, adenosine diphosphate.

ACKNOWLEDGMENT

We thank M. P. Martín-Lopez and C. Alcalde-Eon for their assistance in the synthesis of the vitisins A.

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Received for review November 19, 2003. Revised manuscript received March 26, 2004. Accepted April 2, 2004. Financial support provided by MCYT and FEDER program (Spain) and BBSRC (U.K.). The Ph.D. studentship of M.G.A. is supported by a grant from the Ministerio de Educación, Cultura y Deporte (FPU grant), Spain.

JF035360V