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Vascular bioactivity of anthocyanins and phenolic acid degradants: modulation of superoxide and nitric oxide

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Anthocyanins, a sub-class of the flavonoid family of phenolic phytochemicals, may have significant vasoprotective activity[1], but their low bioavailability suggests bioactivity could be mediated by degradation products or metabolites[2]. The present study aimed to investigate vascular bioactivity of selected anthocyanins and phenolic degradants, and potential synergy between flavonoids and ascorbic acid. Bioactivity was assessed by human umbilical vein endothelial cell (HUVEC) superoxide production and expression of NAD(P)H oxidase (NOX), and production of nitric oxide (NO) and expression of endothelial NO synthase (eNOS).

Cell viability was measured by the WST-1 assay (Roche Applied Science, UK), and superoxide production assessed based on previously reported methods[3]. Briefly, HUVECs were incubated with 0.1 μM angiotensin II (Ang II), with or without treatment compounds (0.1–10 μM), for 6 h and superoxide production quantified by reduction of ferricytochrome c. NOX expression was investigated by immunoblotting, and commercially available kits were used to quantify nitric oxide (NO) production (Nitrite/Nitrate Assay, Cayman Chemical Company, USA) and eNOS expression (Quantikine, R&D Systems, UK).

Cell viability was not affected ($P>0.05$) by any treatment at 10 μM (data not shown). Superoxide production (as tabulated below) was significantly decreased ($P<0.05$) by the anthocyanins peonidin- and malvidin-glucoside; and the anthocyanin phenolic degradants protocatechuic acid, vanillic acid, and syringic acid. Superoxide was also significantly decreased by protocatechuic acid in combination with two flavonoids, epicatechin and quercetin, and ascorbic acid; although to a lesser extent than with protocatechuic acid alone.

	Superoxide production (% of control)					
	0.1 μM		1 μM		10 μM	
Treatment	Mean	SD	Mean	SD	Mean	SD
Cyanidin-glucoside	256.64*	3.66	287.26*	8.63	75.96	15.59
Peonidin-glucoside	78.76*	8.17	95.75	0.69	59.65*	9.60
Malvidin-glucoside	88.86	8.09	84.19	5.31	12.31*	13.95
Protocatechuic acid	111.27	5.15	116.35	13.21	21.40*	45.05
Vanillic acid	72.24	24.99	61.77*	10.07	71.15	8.83
Syringic acid	125.44	19.44	46.58*	23.50	95.73	4.20
Cyanidin-glucoside combination†	109.78	7.77	81.34	31.76	68.63	54.24
Protocatechuic acid combination†	79.26	19.94	83.60	16.42	62.25*	10.25

†Cyanidin-glucoside or protocatechuic acid with epicatechin, quercetin, and ascorbic acid (equimolar ratio).
*Significant difference versus Ang II stimulated control ($P<0.05$; ANOVA with Tukey post-hoc; $n = 3$).

NOX expression was not significantly altered by anthocyanin degradants (data not shown). No significant modulation of NO decomposition products was observed, however cyanidin-, peonidin- and petunidin-glucosides significantly ($P<0.05$) upregulated eNOS (data not shown). In conclusion, anthocyanins and their phenolic degradants decreased superoxide production, whilst only anthocyanins upregulated eNOS, suggesting differential bioactivity of anthocyanins and degradants. Given the low bioavailability of anthocyanins *in vivo*, their phenolic degradants may enhance vascular function by decreasing superoxide production and thus NO scavenging, as opposed to direct stimulation of NO production.

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