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Delphinidin inhibits endothelial cell proliferation and cell cycle progression through a transient activation of ERK-1/-2

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Abstract

Epidemiological studies have shown that a diet rich in fruits and vegetables might reduce the risk of cardiovascular diseases and protect against cancer by mechanisms that have not been elucidated yet. This study was aimed to define the effect of delphinidin, a vasoactive polyphenol belonging to the class of anthocyanin, on bovine aortic endothelial cells (BAECs) proliferation. Delphinidin inhibited serum- and vascular endothelium growth factor-induced BAECs proliferation. This antiproliferative effect of delphinidin, is triggered by ERK-1/-2 activation, independent of nitric oxide pathway and is correlated with suppression of cell progression by blocking the cell cycle in G_0/G_1 phase. Furthermore, suppression of cell cycle progression is associated with the modulation of the mitogenic signaling transduction cascade. This includes over-expression of caveolin-1 and $p21^{WAF1/Cip1}$ and down-expression of Ras and cyclin D1. In conclusion, the antiproliferative effect of delphinidin may be of importance in preventing both plaque development and stability in atherosclerosis and tumor dissemination in cancer.

Keywords: Anthocyanins; Endothelial cell proliferation; Cell cycle; ERK-1/-2; Caveolin-1

1. Introduction

Anthocyanins are a subclass of polyphenols that are widely spread in food of vegetal origin. In long overdue studies, it was shown that such compounds may have potentially beneficial effects on various pathologies by reducing the risk of cardiovascular diseases, by possessing anti-inflammatory and chemoprotective properties [1]. The exact mechanism(s) by which they act has not been totally elucidated but may be related to their ability to suppress cell proliferation and migration, tumor cell invasion or angiogenesis [2–4]. They have also been reported to scavenge reactive oxygen species, inhibit oxidation of low-density lipoprotein, prevent platelet aggregation, and interact with the nitric oxide (NO) generating pathway [5–7]. Delphinidin is a polyphenol belonging to the group of anthocyanin. Previous studies from our laboratory have clearly shown that delphinidin elicits an endothelium-dependent relaxant effect [6]. This action depends on the ability of delphinidin to stimulate NO production through an increase in cytosolic calcium, independently of its anti-oxidant property [8]. In addition, delphinidin has been recently reported to inhibit the growth of human tumor cell line by shutting off the epidermal growth-factor receptor downstream signaling cascade [9]. Thus, delphinidin may represent a promising class of compound in a view of chemoprevention.

Under physiological state, endothelial cells normally remain quiescent, show a low turnover rate and rarely proliferate [10]. Once they received an appropriate stimulus, endothelial cells rapidly proliferate and form new blood vessels by a process called angiogenesis [11]. Although proliferation of vascular endothelial cells plays a critical role in normal biological processes such as embryonic development or wound healing [12], uncontrolled proliferation and subsequent angiogenesis is found in pathological conditions such as atherosclerosis or tumor

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Abbreviations: BAECs, bovine aortic endothelial cells; NO, nitric oxide; VEGF, vascular endothelium growth factor; e-NOS, endothelial NO-synthase; $[Ca^{2+}]_i$, cytosolic free calcium; L-NA, N^{ω}-nitro-L-arginine; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

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formation [12]. These data are supported by the fact that increased endothelial cell proliferation and neovascularization may increase endothelium permeability which not only promote atherogenesis, but also accelerate plaque progression and maintain tumor growth [13,14]. Therefore, regulation of endothelial cell proliferation may be an alternative therapeutic option for the treatment of certain cardiovascular diseases or cancers.

Homeostasis of the vascular endothelium, both in terms of metabolic and physiological activities, is subjected to a fine tuning by individual nutriments or their derivatives. We therefore investigated the effects of delphinidin on proliferation, cell cycle progression and related signaling pathways in cultured bovine aortic endothelial cells (BAECs). We found that delphinidin reduced both serum- and vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation by perturbing progression through G_0/G_1 to S phase of the cell cycle. This anti-proliferative effect of delphinidin is independent of the NO pathway but required the activation of ERK-1/-2 pathway. Furthermore, during the suppression of cell cycle progression upon treatment with delphinidin, endothelial cells displayed changes in protein expression of the mitogenic signaling transduction cascade. Indeed, delphinidin treatment elicited over-expression of caveolin-1 and $p21^{WAF1/Cip1}$ and down-expression of Ras and cyclin D1.

2. Materials and methods

2.1. Cell culture conditions

BAECs were grown as described previously [8]. Briefly, cells were cultured at 37° in 5% CO_2 atmosphere in plastic flasks (Nunc) precoated with type I collagen (0.06 mg/mL) in a mixture of DMEM and Ham's F12 (1:1, v/v) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 mg/mL heparin, antibiotics (100 U/mL penicillin and 100 U/mL streptomycin), 2.5 μg/mL fungizone and 10 μM Vitamin C (BioWhittaker). Cultures were used up to fifth passages. For western blot, cells were subcultured in 60 mm Petri dishes (Nunc).

2.2. Cell proliferation assay

BAECs were seeded in 96-well plates coated with type I collagen (0.06 mg/mL) at low density to avoid contact inhibition during the experiment (3000 cells per well). Four hours after plating, cells were arrested by culturing for 24 hr in absence of FBS, which was sufficient to accumulate cells in G_0/G_1 phase. BAECs were then incubated 24, 48 or 72 hr with the experimental medium containing 2% FBS. Cell number was determined by a colorimetric assay using CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay from Promega.

2.3. Cell cycle analysis

BAECs were seeded in 35 mm Petri dishes (Nunc) at 90,000 cells per well in total culture medium. Four hours after plating, cells were arrested by culturing them in absence of FBS for 24 hr. Growth arrested BAECs were stimulated with 2% FBS-containing medium alone or in the presence of delphinidin during 18 hr. This period of incubation has been chosen to arrest cells before the time needed for a complete cell cycle period. After treatment, cells were fixed in 70% ethanol during at least 1 hr at 4°. Cells were washed in Hank's balanced salt solution before resuspension in a solution containing type I RNase A (0.1 mg/mL) and were incubated for 10 min at 37°. Propidium iodide (PI, 0.5 mg/mL) was then added and samples were allowed to stand 30 min in the dark at room temperature before flow cytometry analysis using CELLQuest software (Becton Dickinson).

2.4. Western blot analysis

After treatment, cells were harvested and lysed for 1 hr in 200 μL of ice-cold lysis buffer (in mM: 50 Tris, 250 NaCl, 8 $MgCl_2$, 5 EDTA, 0.5 EGTA, 100 NaF, 2 sodium orthovanadate, 1 PMSF supplemented with 1% Triton X-100 and 10 μg/mL of aprotinin, leupeptin and pepstatin). After quantification by the Bradford method, 50 μg proteins was resuspended in Laemmli's buffer, separated on 7 or 12% SDS–PAGE. Blots were probed with e-NOS (1/2000), caveolin-1 (1/10,000), Ras (1/2000), phophorylated-ERK-1/-2 (1/10,000), cyclin D1 (1/250), $p21^{WAF1/Cip1}$ (1/250), followed by the horseradish peroxidase-conjugated antibody (1/50,000). Blots were treated with enhanced chemiluminescence reagents for 10 min and exposed to CL-Xposure films (Kodak). Films were scanned and densitometric analysis was realized on the scanning images using Scion Image-Release Beta 4.02 software (http://www.scioncorp.com). Results were expressed as the percentage of protein staining vs. basal expression in control cells. The sample loading was verified by staining membranes with Ponceau red and amido black, and reprobing it with β-actin (1/50,000) (data not shown).

2.5. Statistical analysis

Results are expressed as mean ± SEM of N separate experiments. Analysis of variance (ANOVA) or unpaired Student's *t*-test were used for statistical analysis with $P < 0.05$ being considered significant.

2.6. Reagents

The following reagents were used: delphinidin chloride (Extrasynthèse), VEGF (Cell concept), PD98059 (Tocris), N^{ω}-nitro-L-arginine (Sigma), U0126 (Calbiochem). The following antibodies were used: endothelial nitric oxide

synthase (e-NOS), caveolin-1 and cyclin D1 (clone 3, clone 2297 and clone DCS-6, respectively, BD Biosciences), Ras (clone RAS10, Upstate Biotechnology), phophorylated-ERK-1/-2 and β-actin (clone MAPK-YT, Sigma), $p21^{WAF1/Cip1}$ (clone C-19, Santa Cruz Biotechnology) and horseradish peroxidase (HRP)-conjugated antibody (Promega).

3. Results

3.1. Delphinidin reduces serum- and VEGF-induced proliferation of BAECs

Preliminary studies showed that delphinidin was able to reduce in concentration- and time-dependent manner basal BAECs proliferation. The effect of delphinidin was maximal at 10^{-1} g/L and 72 hr of treatment for the concentration and the incubation period, respectively (not shown). However, for the following studies, delphinidin was used at 10^{-2} g/L, a maximally active concentration that promote endothelium-dependent relaxation of rat aortic rings [6], the increase in $[Ca^{2+}]_i$ in endothelial cells [8], the protection of endothelial cells against apoptosis [15] and the inhibition of epidermal growth-factor receptor in human vulva carcinoma cell line A431 [9]. As illustrated in Fig. 1, exposure of BAECs to 10^{-2} g/L delphinidin during 72 hr induced a significant inhibition of basal endothelial cell proliferation in the presence 2% FBS (i.e. 18 ± 2% reduction, N = 16, Fig. 1).

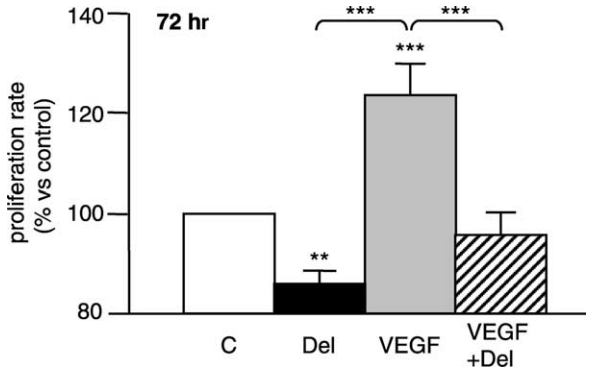

Fig. 1. Delphinidin impairs proliferation of BAECs. Cells were exposed to either control medium (containing 2% FBS), 10^{-2} g/L delphinidin (Del) or 50 ng/mL of VEGF alone or in combination with delphinidin. Values are the mean ± SEM of 12 experiments. Proliferation rate was determined as indicated in Section 2. (**) $P < 0.01$ and (***) $P < 0.001$ significantly different to control.

Incubation of BAECs with 50 ng/mL VEGF, induced a time dependent increase of endothelial cell proliferation with a maximal of 25 ± 7% being reached after 72 hr of treatment. The stimulating effect of VEGF was completely prevented by delphinidin (N = 12, Fig. 1).

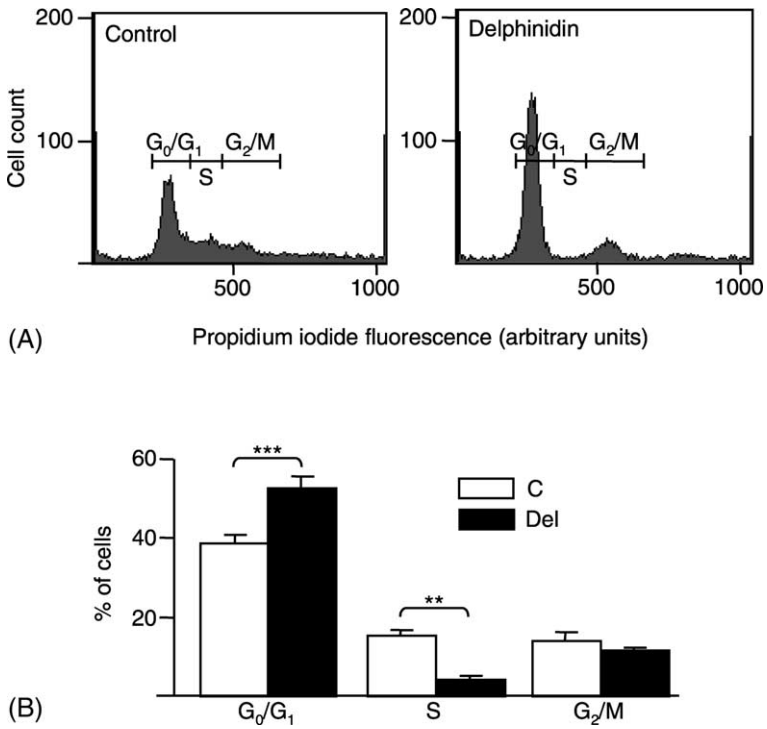

Fig. 2. Delphinidin impairs cell cycle progression of BAECs. Cells were exposed to either control medium (containing 2% FBS) and 10^{-2} g/L of delphinidin during 18 hr and treated as indicated in Section 2. (A) Representative cytometric profiles showing cells in the G_0/G_1, S and G_2/M phases of the cell cycle. (B) Histograms show the percentage of cells in the G_0/G_1, S and G_2/M phases determined by flow cytometric analysis. (**) $P < 0.01$ and (***) $P < 0.001$ significantly different to control.

3.2. Delphinidin impairs cell cycle progression through blocking G_1/S transition

To investigate whether delphinidin-induced cell growth inhibition might be related to alterations in cell cycle, the effect of delphinidin on cell cycle distribution was evaluated. Cytometric profile showed (Fig. 2A) that delphinidin induced an accumulation of BAECs in G_0/G_1 phase associated with a diminution of the S phase without an alteration of the M phase of the cell cycle. Histograms showed an increase of $37.9 \pm 10.5\%$ of cells in G_0/G_1 and a concomitant reduction of 67.4 ± 9.3 and $18.5 \pm 12.0\%$, of cells in S and G_2/M, respectively ($N = 7$, Fig. 2B). It should be noted that the alteration of cell cycle progression induced by delphinidin was independent of apoptosis induction in BAECs (2.3 ± 0.6 and $3.2 \pm 0.8\%$ apoptosis in control and delphinidin-treated cells, respectively, $N = 7$). The lack of apoptosis induction by delphinidin in BAECs has also previously been reported using TUNEL assay and caspase-3 activity measurements [15].

3.3. Delphinidin inhibits BAECs proliferation through NO-independent and ERK-1/-2 kinase-dependent pathways

NO and MAP kinases, especially ERK-1/-2, pathways have been shown to play a role in the regulation of endothelial cell proliferation [16,17].

The nonselective NO-synthase inhibitor, N^{ω}-nitro-L-arginine (L-NA, 200 μM) had no effect on endothelial cell proliferation and did not modify the inhibitory effect of delphinidin ($N = 7$, Fig. 3). Identical results were observed with another nonselective NO-synthase inhibitor, N^{ω}-nitro-L-argine-methyl ester (100 and 200 μM, not shown).

Turning to ERK-1/-2 pathway, treatment of cells with the specific inhibitor of MEK-ERK pathway, U0126 (10 μM), at a concentration at which it had no effect by itself, totally abolished the antiproliferative effect of delphinidin ($N = 9$, Fig. 4A). Similar effects were observed with another specific inhibitor of MEK-ERK pathway, PD98059 (not shown).

In order to confirm the involvement of the ERK-1/-2 pathway in the antiproliferative effect of delphinidin, a time course of ERK-1/-2 activation has been assessed by western blot. As shown in Fig. 4B, delphinidin activated ERK-1/-2 after 10 min of stimulation, but it significantly inhibited basal ERK-1/-2 activity at 1, 6 and 24 hr of stimulation ($N = 3$, Fig. 4B). Finally, delphinidin failed to alter ERK-1/-2 activity after 48 and 72 hr treatments. These results suggest that early activation of ERK-1/-2 may play a trigger role for the antiproliferative effect of delphinidin in BAECs.

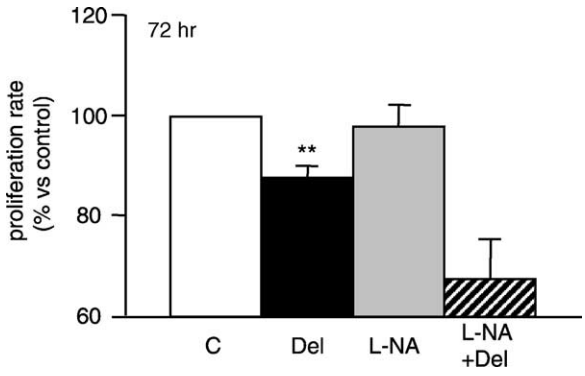

Fig. 3. Delphinidin inhibit BAECs proliferation through NO-independent pathway. BAECs were exposed to either 10^{-2} g/L of delphinidin, 200 μM L-NA or a combination of delphinidin and L-NA. Values are the mean ± SEM of seven experiments. Proliferation rate was determined as indicated in Section 2. (**) $P < 0.01$ significantly different to control.

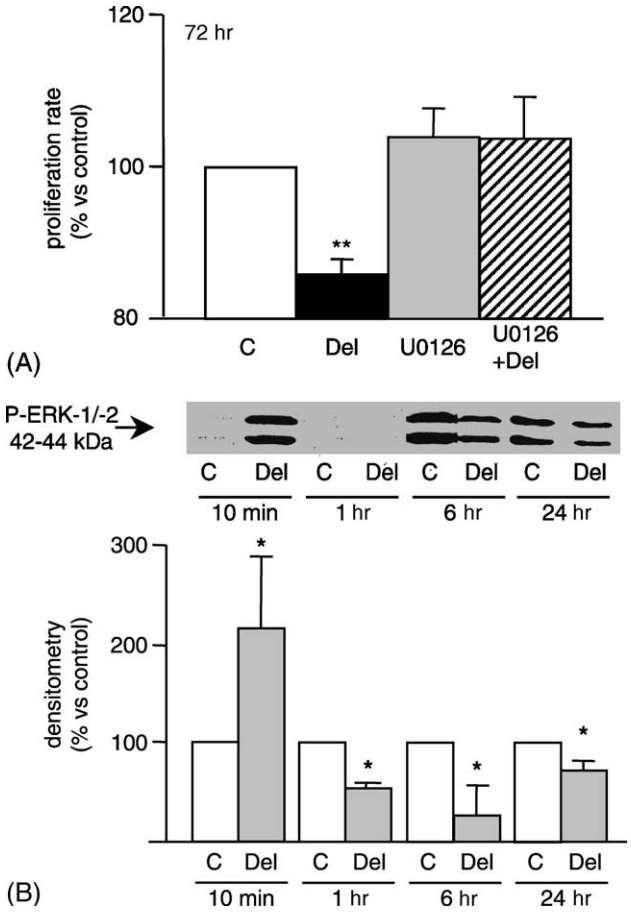

Fig. 4. Delphinidin inhibit BAECs proliferation through MAP kinase-dependent pathway. (A) BAECs were exposed to either 10^{-2} g/L of delphinidin, 10 μM U0126 or delphinidin plus U1026. Proliferation rate was determined as indicated in Section 2. Histograms show the percentage of proliferation induced by delphinidin in absence and in presence of the inhibitors. Values are the mean ± SEM of nine experiments. Proliferation rate was determined as indicated in Section 2. (**) $P < 0.01$ significantly different to control. (B) Western blot of phospho-ERK-1/-2 in BAECs exposed to either control medium (containing 2% FBS) or 10^{-2} g/L of delphinidin during indicated time. Histograms show densitometric analysis of phospho-ERK-1/-2 expression. Data represent the mean ± SEM of 3–12 separate experiments. (*) $P < 0.05$ significantly different to control.

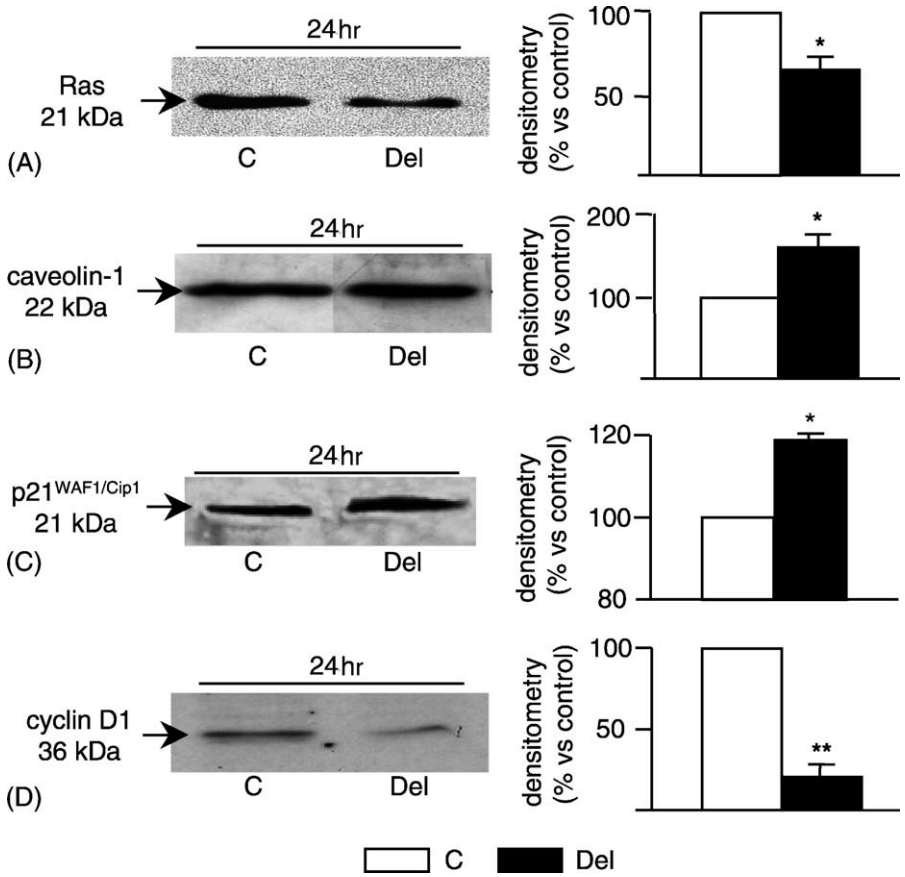

Fig. 5. Delphinidin modulate signaling transduction cascade molecules. BAECs were exposed to either control medium (containing 2% FBS) or 10^{-2} g/L of delphinidin during 24 hr and western blotted to reveal the expression of (A) Ras, (B) caveolin-1, (C) $p21^{WAF1/Cip1}$, and (D) cyclin D1. Histograms show densitometric analysis of protein expression. Data represent the mean ± SEM of 3–15 separate experiments. (∗) $P < 0.05$ and (∗∗) $P < 0.01$ significantly different to control.

3.4. Delphinidin reduces the expression of Ras and induces an over-expression of caveolin-1

We also investigated the expression of two proteins implicated in the primary transduction cascade of the mitogenic signal, Ras and caveolin-1 [18]. Western blot analysis showed that treatment of BAECs during 24 hr with delphinidin induced a significant reduction of Ras expression from 36.4 ± 7.9% (N = 4, Fig. 5A).

Conversely, treatment of BAECs with delphinidin induced a time dependent increase of caveolin-1 expression being 44.5 ± 10.1 and 93.7 ± 10.8% at 24 and 48 hr treatment, respectively (N = 3–15, Fig. 5B).

3.5. Delphinidin elicits a down-regulation of cyclin D1 expression and an over-expression of $p21^{WAF1/Cip1}$

We also assessed the effect of delphinidin treatment on the regulation and the expression of one of the major cyclin operative in G_1/S transition by western blotting $p21^{WAF1/Cip1}$ and cyclin D1. Analysis revealed that incubation of BAECs with delphinidin during 24 hr, induced an increase of $p21^{WAF1/Cip1}$ expression (18.8 ± 1.6%, N = 3, Fig. 5C), and a significant inhibition of cyclin D1 expression (79.9 ± 7.7% of inhibition vs. control cells, N = 3, Fig. 5D). The inhibitory effect of delphinidin on cyclin D1 expression has also been detected earlier (18 ± 0.9% of inhibition vs. control cells after 1 hr of stimulation by delphinidin, N = 3, not shown).

4. Discussion

The present study was designed to test whether delphinidin, a polyphenol present in human diet, may exert cardioprotection and chemoprotective properties. Here we reported that delphinidin inhibits both serum- and VEGF-induced proliferation of BAECs, by cell cycle progression arrest in G_0/G_1 phase. The antiproliferative effect was independent of the NO pathway but was triggered by the activation of ERK-1/-2 pathway. We also reported alterations of the mitogenic signal transduction reflected by an inhibition of Ras expression, an over-expression of caveolin-1, an induction of $p21^{WAF1/Cip1}$ expression and finally the down-regulation of cyclin D1. Altogether, the antiproliferative effects of delphinidin represent potential mechanisms for prevention of cardiovascular diseases and cancers, in which endothelial cell

proliferation favors atherosclerotic plaque development and/or fragility and tumor metastasis.

As revealed by flow cytometry, the antiproliferative effect of delphinidin is associated with an accumulation of cells in G_0/G_1 phase of the cycle. Because there was no evidence of apoptosis induction during treatment of BAECs by this compound, the observed accumulation in G_0/G_1 reflected a specific effect of delphinidin on cell cycle progression rather than a decrease of cell number due to apoptosis. We therefore hypothesized that the antiproliferative properties of delphinidin are causally related to the modulation of signaling cascade involved in cell cycle progression and proliferation.

Although several angiogenic activators, including growth factors which are present in serum, are known to induce cell proliferation by activating ERK-1/-2 pathway [19], such kinases may also exert cell growth inhibitory properties as described recently by Sipeki *et al*. [17]. These authors reported that the antiproliferative effect of phorbol esters is abolished in presence of specific inhibitors of ERK pathway. In accordance with these findings, inhibition of serum-induced BAECs proliferation by delphinidin is totally abolished in presence of U0126. It should be noted that U0126 at used concentration, had no effect on endothelial cell proliferation suggesting that it specifically inhibited the antiproliferative effect of delphinidin. Since delphindin induced the activation of ERK-1/-2 only at 10 min but not a longer time exposure, this pathway may play a triggering role in inhibiting BAECs proliferation.

Recently, we have reported that delphinidin can activate ERK-1/-2 pathway resulting in an over-expression of e-NOS expression leading to protection of BAECs against apoptosis [15]. Also, such an increase in e-NOS expression has been reported to be able to inhibit cell proliferation [20], we reported here that NO synthase inhibitors heightened the antiproliferative effect of delphinidin. The involvement of NO in endothelial cells proliferation has already been observed in response of VEGF or substance P [21,22]. In accordance with these studies, our results suggest that the NO pathway, activated either by serum or delphinidin itself, as reported previously [6], acts as a proliferative agent. Thus, inhibition of NO pathway unmasks the antiproliferative effect of delphinidin and reveals an NO-independent antiproliferative pathway of delphinidin. These results also shed light divergent downstream targets after ERK-1/-2 activation by delphinidin in BAECs. On one hand, ERK-1/-2 pathway is linked to over-expression of e-NOS and thus NO production in the anti-apoptotic effect of delphindin [15], on the other hand ERK-1/-2 independently of NO is involved for the antiproliferative of this compound.

In endothelial cells, lipid-modified signaling molecules such as src-like kinases, H-Ras, endothelial NO-synthase and MAP kinases, are concentrated within caveolae membranes [23]. The direct interaction of caveolin family members with signaling molecules leads to their inactivation [23]. Engelman and coworkers [18,24] have previously shown that the induction of caveolin-1 expression blocks cell proliferation by directly inhibiting the Ras/ERK-1/-2 signaling cascade in NIH-3T3 cells. This negative regulatory effect of caveolin-1 on proliferation was also reported in endothelial cells [25]. In accordance with this study, we found that treatment of cells with delphinidin induced a down-regulation of Ras expression and an induction of caveolin-1 expression. It should be noted that the enhanced expression of caveolin-1 was observed at a time at which delphinidin rather decreased than increased ERK-1/-2 activation (i.e. 24 hr). These results suggest that endothelial cell proliferation might be regulated in a sequential manner. Thus, ERK-1/-2 activation as a trigger is involved at the early stage whereas enhanced expression of caveolin-1 took place at late stage of the regulation of endothelial cell proliferation. However, further studies are needed to better understand the precise link between ERK-1/-2 and caveolin-1 expressions. Nevertheless, delphinidin can modulate both ERK-1/-2 activity and Ras and caveolin-1 expressions in BAECs.

Finally in eukaryotes, the passage through the cell cycle is orchestrated by the function of a family of protein kinase composed of a catalytic subunit, the cdk and its essential activating partner, the cyclin [26]. Under normal conditions, these complexes are activated at specific intervals through a series of events which results in progression of cells through different phases of cell cycle. During the progression in cell cycle, the cdk–cyclin complexes are inhibited *via* the binding to ckis (inhibitors of cdk) such as the Cip/kip family of proteins [27]. We focused on the effect of delphinidin on cell cycle regulatory molecules operative in G_1 phase and during the G_1/S transition. The results showed that delphinidin induced a significant up-regulation of $p21^{WAF1/Cip1}$ expression and a dramatic decrease of cyclin D1 expression, the major cyclin operative in G_1/S transition. Inhibition of cell cycle progression through the induction of p53 and $p21^{Cip/kip}$ has been reported to be under control of both ERK-1/-2 and caveolin-1 [17,28,29]. It may be possible that delphinidin through Ras/ERK-1/-2 and caveolin-1 may affect the regulatory components of cell cycle such as $p21^{WAF1/Cip1}$ and cyclin-1. Modulation of these regulatory molecules leads to cell cycle progression arrest in G_0/G_1 phase and thus inhibition of BAECs proliferation.

In conclusion, delphinidin exerts antiproliferative effect on basal and VEGF-induced proliferation of BAECs. This beneficial property of delphinidin may be of importance in atherosclerosis in which proliferation is an important determinant of atherosclerotic plaque development and stability. As proliferation is also a major step in angiogenesis, it can facilitate tumor dissemination and growth. Therefore, these results give also credence to the notion that delphinidin may be a potential cancer chemopreventive agent.

Acknowledgments

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References

[1] Middleton Jr E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673–751.
[2] Nagase H, Sasaki K, Kito H, Haga A, Sato T. Inhibitory effect of delphinidin from Solanum melongena on human fibrosarcoma HT-1080 invasiveness in vitro. Planta Med 1998;64:216–9.
[3] Chen S, Ruan Q, Bedner E, Deptala A, Wang X, Hsieh TC, Traganos F, Darzynkiewicz Z. Effects of the flavonoid baicalin and its metabolite baicalein on androgen receptor expression, cell cycle progression and apoptosis of prostate cancer cell lines. Cell Prolif 2001;34:293–304.
[4] Fotsis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H, Wahala K, Montesano R, Schweigerer L. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 1997;57:2916–21.
[5] Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J Agric Food Chem 2002;50:166–71.
[6] Andriambeloson E, Magnier C, Haan-Archipoff G, Lobstein A, Anton R, Beretz A, Stoclet JC, Andriantsitohaina R. Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. J Nutr 1998;128:2324–33.
[7] Freedman JE, Parker III C, Li L, Perlman JA, Frei B, Ivanov V, Deak LR, Iafrati MD, Folts JD. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 2001;103:2792–8.
[8] Martin S, Andriambeloson E, Takeda K, Andriantsitohaina R. Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. Br J Pharmacol 2002;135:1579–87.
[9] Meiers S, Kemeny M, Weyand U, Gastpar R, von Angerer E, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. J Agric Food Chem 2001;49: 958–62.
[10] Denekamp J. Vascular attack as a therapeutic strategy for cancer. Cancer Metastasis Rev 1990;9:267–82.
[11] Risau W. Mechanisms of angiogenesis. Nature 1997;386:671–4.
[12] Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992;267:10931–4.
[13] McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, Naylor AR, Brindle NP. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg 1999;30:261–8.
[14] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249–57.
[15] Martin S, Giannone G, Martínez MC, Andriantsitohaina R. Anti-apoptotic affect of an anthocyanin, delphinidin, is mediated on a cyclic-GMP increase and NO dependent pathway. Thromb Haemost 2001;86(Suppl):446.
[16] RayChaudhury A, Frischer H, Malik AB. Inhibition of endothelial cell proliferation and bFGF-induced phenotypic modulation by nitric oxide. J Cell Biochem 1996;63:125–34.
[17] Sipeki S, Bander E, Ways DK, Farago A. Activation of Erk1/Erk2 and transiently increased p53 levels together may account for p21 expression associated with phorbol ester-induced transient growth inhibition in HepG2 cells. Cell Signal 2002;14:115–21.
[18] Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T, Kohtz DS, Lisanti MP. Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. FEBS Lett 1998;428:205–11.
[19] Jones MK, Sarfeh IJ, Tarnawski AS. Induction of in vitro angiogenesis in the endothelial-derived cell line, EA hy926, by ethanol is mediated through PKC and MAPK. Biochem Biophys Res Commun 1998;249:118–23.
[20] Chen L, Daum G, Forough R, Clowes M, Walter U. Overexpression of human endothelial nitric oxide synthase in rat vascular smooth muscle cells and in balloon-injured carotid artery. Circ Res 1998;82:862–70.
[21] Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, Ziche M. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am J Physiol 1996;270:H411–5.
[22] Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, Ledda F. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. J Clin Invest 1994;94:2036–44.
[23] Smart EJ, Graf GA, McNiven MA, Sessa WC, Engelman JA, Scherer PE, Okamoto T, Lisanti MP. Caveolins, liquid-ordered domains, and signal transduction. Mol Cell Biol 1999;11:7289–304.
[24] Engelman JA, Wykoff CC, Yasuhara S, Song KS, Okamoto T, Lisanti MP. Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. J Biol Chem 1997;272:16374–81.
[25] Liu J, Razani B, Tang S, Terman BI, Ware JA, Lisanti MP. Angiogenesis activators and inhibitors differentially regulate caveolin-1 expression and caveolae formation in vascular endothelial cells. Angiogenesis inhibitors block vascular endothelial growth factor-induced down-regulation of caveolin-1. J Biol Chem 1999;274:15781–5.
[26] Jacks T, Weinberg RA. Cell-cycle control and its watchman. Nature 1996;381:643–4.
[27] Sherr J, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 1995;9:1149–63.
[28] Galbiati F, Volonte D, Liu J, Capozza F, Frank PG, Zhu L, Pestell RG, Lisanti MP. Caveolin-1 expression negatively regulates cell cycle progression by inducing G(0)/G(1) arrest via a p53/p21(WAF1/Cip1)-dependent mechanism. Mol Biol Cell 2001;12:2229–44.
[29] Agarwal ML, Taylor WR, Chernov MV, Chernova OB, Stark GR. The p53 network. J Biol Chem 1998;273:1–4.