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Cyanidin-3-O-β-glucoside with the aid of its metabolite protocatechuic acid, reduces monocyte infiltration in apolipoprotein E-deficient mice

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ABSTRACT

Polyphenols, including anthocyanins, from various plant foods are effective in reducing the severity of atherosclerosis in animal and human studies. Due to the poor understanding of the bioavailability of anthocyanins, the potential antiatherogenic mechanisms underlying the action remain largely unknown. Herein, we found that oral gavage of cyanidin-3-O-β-glucoside (Cy-3-G) could be transformed into protocatechuic acid (PCA), and the plasma maximal levels of Cy-3-G were 3.7-fold lower than that of PCA in the apolipoprotein E (ApoE)-deficient mice. Subsequently, we observed that PCA treatment has a higher capacity than Cy-3-G treatment in decreasing CC chemokine receptor 2 (CCR2) expression in the mouse peripheral blood monocytes (PBMs), along with reducing the mouse PBMs chemokine toward CC ligand-2 (CCL2) in a Boyden chamber. Interesting, in the ApoE-deficient mouse model, orally gavaged with Cy-3-G has a higher ability than gavaged with PCA to reduce CCR2 expression in PBMs. PBMs deprived from the Cy-3-G-treated ApoE-deficient mice have a lower ability than those from PCA-treated animals to migrate toward CCL2. Furthermore, as compared with the PCA group, Cy-3-G treatment more efficiently reduced thioglycollate-induced macrophage infiltration into the abdominal cavity. Thus, we suggest that Cy-3-G may reduce the monocyte infiltration in mice via down-regulation of CCR2 expression in monocytes, at least in part, with the aid of its metabolite PCA. These above data imply that the anti-monocyte/macrophage infiltration property of Cy-3-G and its metabolite PCA may be an important antiatherogenic mechanism for anthocyanins.

1. Introduction

Polyphenols represent a group of secondary metabolites that exist widely in plant foods. Extensive epidemiological studies have shown that dietary polyphenol consumption is inversely associated with the incidence of atherosclerotic diseases [1]. Experimental animal work has also shown the antiatherogenic properties of polyphenols from various sources [2]. Furthermore, both in vitro cell culture studies and experimental animal work have been utilized to explore mechanisms underlying the protective effect of these native therapeutic agents [3]. Among the potential mechanisms revealed to date, the anti-inflammatory effect of polyphenols has drawn our attention intensively [4].

Anthocyanin, the flavonoid consumed by human beings [5], is most abundant in various colorful fruits, vegetables, red wine and grains [6]. Numeral data from population and animal studies indicated that anthocyanins are antiatherogenic natural agents [7–10]. Our group have performed a series of studies using anthocyanin-rich black rice and anthocyanin-rich extract, and demonstrated that anthocyanins play a protective role in reducing the severity or risk of atherosclerosis [7–9]. These along with the observations made by other groups [10], also demonstrated that anthocyanin-rich extract are antiatherogenic in vivo.

Previous studies have shown that anthocyanins could be absorbed in intact glycosylated forms and were detected in the plasma and urine of human subjects and animals, but the proportion of anthocyanins intaken is far higher than that of excreted [11,12], indicating that other unidentified metabolites exist. Indeed, Tsuda et al. [13] reported that protocatechuic acid (PCA) was a metabolite of cyanidin-3-O-β-glucoside (Cy-3-G) in rats. Furthermore, a recent human study revealed that after oral administration of Cy-3-G, the plasma maximal levels of PCA are approximately 259-fold higher than that of its parent Cy-3-G, and PCA could account for almost 73% excretion of the ingested Cy-3-G [14]. Thus, it is possible that either parent Cy-3-G or its metabolite PCA or both is responsible for the antiatherogenic effect of anthocyanins.

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In the current study, we demonstrated that oral gavage of Cy-3-G could be transformed into PCA in the apolipoprotein E (ApoE)-deficient mice, and the plasma maximal level of Cy-3-G is 3.7-fold lower than that of PCA. More importantly, we showed that Cy-3-G or PCA treatment at the physiological order of concentrations, attenuated the monocyte chemokine in vitro, and inhibited the macrophage infiltration into the abdominal cavity of the ApoE-deficient mice via negatively regulating CC chemokine receptor 2 (CCR2) expression.

2. Materials and methods

2.1. Materials

RPMI 1640 medium, penicillin-streptomycin, L-glutamine, and fetal bovine serum (FBS) were obtained from Gibco BRL (Grand Island, NY). PCA (purity > 97%), bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-yl)-diphenyl tetrazolium bromide (MTT) were from Sigma–Aldrich (St. Louis, MO). Cy-3-G (purity > 97%) was kindly provided by Polyphenol AS (Sandnes, Norway). HPLC grade acetonitrile and formic acid were obtained from Merck (Darmstadt, Germany). Trizol reagent, M-MLV reverse transcriptase, and PCR Mast Mix were purchased from Invitrogen Life Technology (Carlsbad, CA). Recombinant mouse CC ligand-2 (CCL2) was from R&D Systems (Minneapolis, MN).

2.2. Sample collection and quantification of plasma Cy-3-G and PCA

10-week-old ApoE-deficient mice (Jackson Laboratories, Sacramento, CA) fasted overnight were once orally gavaged with Cy-3-G (25 mg/kg bw), PCA (25 mg/kg bw), or vehicle normal saline. Blood samples were then collected successively from the retro-orbital venous plexus at 0, 0.5, 1, 2, 4, 6, 8 h. Before the measurement of plasma Cy-3-G and PCA, plasma samples were manipulated as previously described [15]. All the animal procedures were approved by the Animal Care and User Committee of Sun Yat-sen University.

An Agilent 1200 series high-performance liquid chromatography coupled to an Agilent 6410 triple quadrupole mass spectrometer (HPLC-MS/MS) and an Agilent Zorbax SB-C18 column (2.1 mm × 50 mm, 1.8 μm) were used to quantify the levels of plasma Cy-3-G and PCA. The solvents were A (5% formic acid in water, v/v) and solvent B (acetonitrile), the gradient elution program was performed as follows: a linear gradient of 2–8% B from 1 to 3 min, followed by a 7 min linear gradient to 10% B, a 5 min linear gradient to the initial condition, and holding for 5 min. Flow rate was 0.2 mL/min, and injection volume was 5 μL.

The MS/MS detection was performed by acquiring data in positive ion mode for Cy-3-G and in negative ion mode for PCA. Electrospray ionisation (ESI) was performed with the following spray chamber conditions: drying gas flow of 8.0 L/min, nebulizer pressure of 40 psi, and drying gas temperature of 350 °C, applying a voltage of 5000 V. Plasma Cy-3-G and PCA levels were quantified by calibration curves obtained with the corresponding standard substances.

2.3. Isolation and culture of mouse peripheral blood monocytes

Mouse peripheral blood mononuclear cells were isolated from the heparinized whole blood obtained from the adult ApoE-deficient or C57BL/6J mice (Jackson Laboratories, Sacramento, CA) by commercially available Ficoll-Paque Plus kit (GE Healthcare, Buckinghamshire, UK). The mouse peripheral blood monocytes (PBMs) were further purified from the peripheral blood mononuclear cells suspension by Percoll Plus (GE Healthcare, Buckinghamshire, UK) and cultured in RPMI 1640 medium supplemented with 10% FBS for 12 h. The viability of mouse PBMs was >98% as assessed by trypan blue exclusion.

2.4. Cell viability assay

The MTT assay was conducted to evaluate the effects of Cy-3-G and PCA on cell viability, as described previously [16]. Briefly, mouse PBMs were seeded on a 96-well plate. Following the treatment of the cells with or without Cy-3-G and PCA for 24 h, MTT was added and incubated for 4 h for the formation of formazan. After the addition of DMSO to dissolve formazan crystals, absorbance of formazan was measured at λ = 570 nm.

2.5. Cy-3-G and PCA treatment

To investigate the effects of Cy-3-G and PCA on CCR2 expression, mouse PBMs were serum-starved for 6 h, and then incubated with indicated concentrations of Cy-3-G or PCA for 24 h.

2.6. Western blotting analysis

Proteins (40 μg) from whole-cell lysates were separated by SDS-polyacrylamide gel electrophoresis. Method for Western blotting has been previously described [17]. Specific antibodies used included CCR2 (cat# ab21667, Abcam, Cambridge, UK), and β-actin (cat# 4967, Cell Signaling Technology, Danvers, MA).

2.7. Quantitative real-time RT-PCR

Total mRNA levels for CCR2 and β-actin in mouse PBMs were quantified using a real-time quantitative RT-PCR (qRT-PCR) assay as previously described [18]. Mouse primer sequences used were as follows: CCR2, 5′-AGAGAGCTGCAGCAAAAAGG-3′ (forward), 5′-GGAAAGAGGCAGTTGCAAAG-3′ (reverse); β-actin, 5′-AGTGTGACGTTGACATCCGTA-3′ (forward), 5′-GCCAGAGCAGTAATCTCCTTCT-3′ (reverse). β-Actin mRNA was used as the internal control.

2.8. In vitro monocyte chemotaxis assay

In vitro monocyte chemotaxis assays were performed in a commercially available 96-well modified Boyden chamber with a 5-μm filter pore size (Neuro Probe Inc., MD). Mouse PBMs suspension was placed on top of the membranes, and medium with or without recombinant mouse CCL2 (10 nmol/L) [19] was added to the bottom feeder wells. After 1.5 h, the upper portion was removed and cell numbers were counted in four random high power 400× fields and expressed as the mean number of cells per field.

2.9. Macrophage infiltration into the abdominal cavity

The macrophage infiltration into the abdominal cavity was evaluated in a thioglycollate-induced peritonitis model, in which the CCR2/CCL2 pathway plays a predominant role in the infiltration of macrophages [20,21]. Male, ten-week-old ApoE-deficient mice were once a day orally gavaged with Cy-3-G (25 mg/kg bw), PCA (25 mg/kg bw), or normal saline for 11 days. At the seventh day, 1 mL of 4% thioglycollate (Sigma–Aldrich, St. Louis, MO) was injected intraperitoneally into mice. After 4 days, the abdominal cavities were washed with pre-warm normal saline, and the number of infiltrated cells was counted in a hemocytometer (Beckman Coulter, Fullerton, CA). Cells were applied to microscope slides with the use of a cytospin centrifuge and stained with Diff-Quik (Fisher Scientific, Schwerte, Germany). Different cell counts were obtained by morphological analysis and determined by counting 300 cells per slide.

2.10. Statistical analysis

Results are presented as the means ± SEM. Data were statistically analysed with either Student's *t* test (2 tailed) or one-way ANOVA coupled with the Student–Newman–Keuls multiple comparison test. Differences were considered significant if $P < 0.05$.

3. Results

3.1. Cy-3-G was metabolized into PCA in the ApoE-deficient mice

HPLC-MS/MS was explored to analyse the concentration of Cy-3-G and its metabolites in plasma obtained from mice gavaged with Cy-3-G and PCA, respectively. The peak 1 (Fig. 1A) was identified as Cy-3-G by the multiple reaction monitoring (MRM) transition *m/z* 449 > 287 for the peak at retention time 11.90 min, consistently with authentic Cy-3-G (Fig. 1B). The peak 2 (Fig. 1A) was identified as PCA by the MRM transition *m/z* 153 > 109 for the peak at retention time 2.14 min, consistently with authentic PCA (Fig. 1C). In the Cy-3-G group, the plasma concentration–time curve showed that at baseline contained neither Cy-3-G nor PCA (Fig. 1D), and Cy-3-G concentration reached the maximum at 0.5 h after the oral gavage (160.4 ± 46.7 nmol/L). The concentration of PCA reached the maximum at 2.0 h (593.8 ± 80.6 nmol/L), which was 3.7-fold higher than Cy-3-G. Subsequently, Cy-3-G and PCA began to gradually decrease and were undetectable in the plasma at 8 h. In the PCA group, Fig. 1D shows

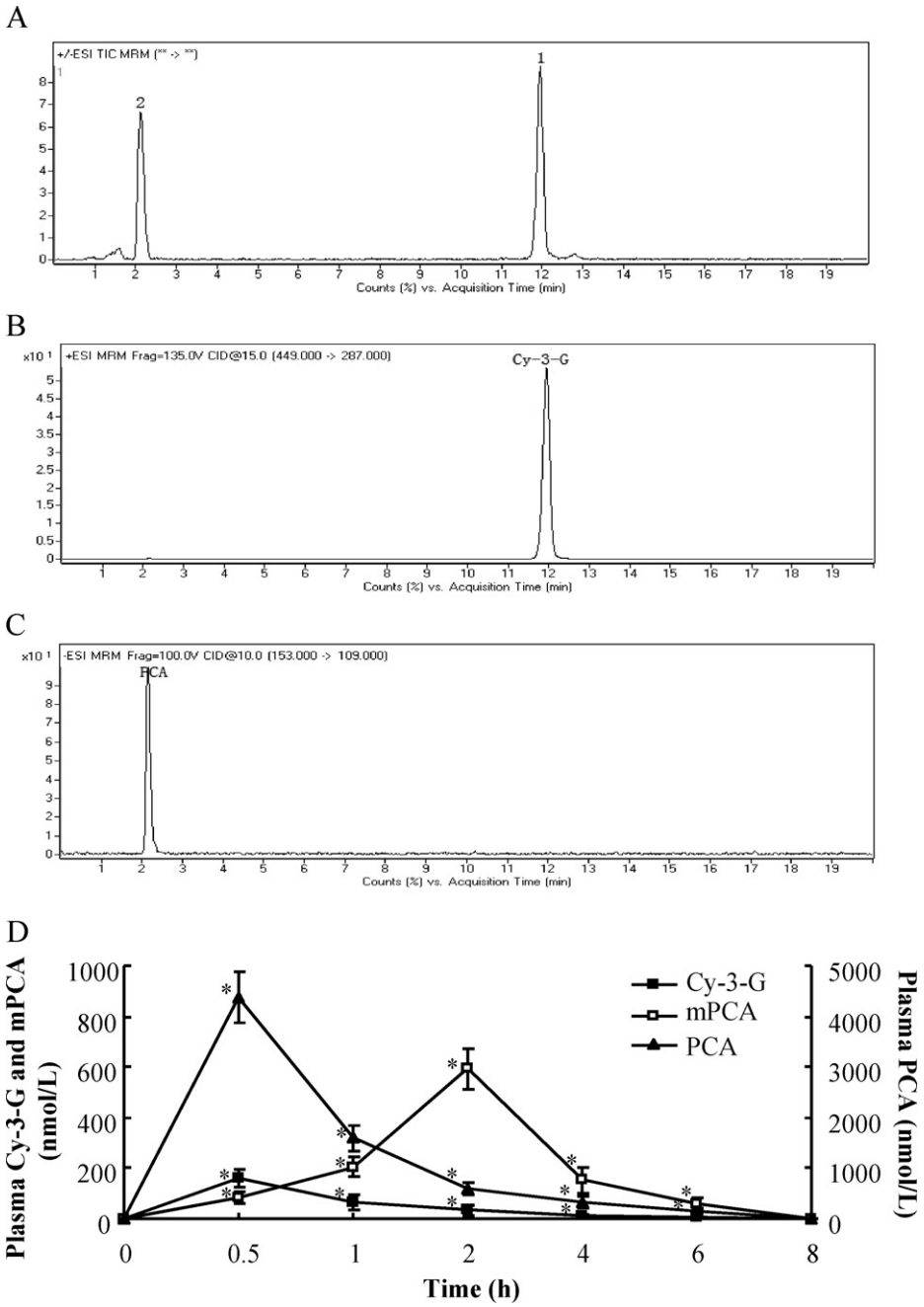

Fig. 1. Plasma concentration of Cy-3-G and PCA in the ApoE-deficient mice. ApoE-deficient mice were once orally gavaged with Cy-3-G (25 mg/kg bw) or PCA (25 mg/kg bw), or its vehicle normal saline. Plasma samples were taken at indicated time for measurement of Cy-3-G and PCA. HPLC-MS/MS profile of plasma samples (A), a solution of authentic Cy-3-G (B), and PCA (C). (D) Plasma concentration–time curves of Cy-3-G, mPCA (metabolite of Cy-3-G) (left axis) and PCA (right axis). Results are means ± SEM ($n = 10$ per group). *Different from baseline, $P < 0.05$.

that plasma PCA concentration reached the maximum at 0.5 h (4380.8 ± 440.6 nmol/L) and was undetectable at 8 h.

3.2. Cy-3-G or PCA reduced CCR2 expression in mouse PBMs

To reveal the possible antiatherogenic mechanism of Cy-3-G and PCA, we isolated PBMs from the ApoE-deficient mice and assessed the effects of Cy-3-G and PCA on CCR2 expression. Incubation of PBMs with Cy-3-G (0.5 μmol/L) or PCA (0.125–0.5 μmol/L) for 24 h significantly decreased CCR2 protein (Fig. 2A) and mRNA (Fig. 2C) expression. To determine whether the inhibitory effect of Cy-3-G or PCA on CCR2 expression is specific to PBMs from the ApoE-deficient mice, we further obtained PBMs from normal C57BL/6J mice. Incubation of PBMs with Cy-3-G (0.125–0.5 μmol/L) or PCA (0.125–0.5 μmol/L) for 24 h significantly decreased CCR2 protein (Fig. 2B) and mRNA (Fig. 2D) expression.

The MTT assays demonstrated that Cy-3-G or PCA treatment at tested concentrations did not significantly affect the viability of PBMs from the ApoE-deficient (Fig. 2E) and C57BL/6J mice (Fig. 2F), suggesting that the attenuation of CCR2 expression by Cy-3-G or PCA was not due to the damage of PBMs.

3.3. Cy-3-G or PCA inhibited mouse PBMs migration in vitro

To investigate whether the decreased expression of CCR2 could lead to a functional consequence, we obtained PBMs from the ApoE-deficient mice and performed a monocyte chemotaxis assay using CCL2 as a chemoattractant in a modified Boyden chamber. As shown in Fig. 3, CCL2 (10 nmol/L) induced a 11.5-fold increase of mouse PBMs chemotaxis in the Boyden chamber. We then pretreated mouse PBMs with different dosages of Cy-3-G or PCA for 24 h, and found that Cy-3-G (0.5 μmol/L) or PCA (0.125–0.5 μmol/L) treatment significantly decreased mouse PBMs migration toward CCL2 (Fig. 3).

3.4. Oral administration of Cy-3-G or PCA decreased CCR2 expression in PBMs of ApoE-deficient mice

To assess whether in vitro observations could extend to in vivo circumstances, the ApoE-deficient mice were once orally gavaged with Cy-3-G (25 mg/kg bw) or PCA (25 mg/kg bw). After 8 h of oral gavage, the change of CCR2 expression in mouse PBMs was then determined. As shown in Fig. 4A and B, the protein and mRNA of CCR2 were significantly reduced in PBMs deprived from Cy-3-G or PCA treated ApoE-deficient mice, as compared with those from the control animals. Consequently, PBMs deprived from Cy-3-G or PCA treated mice exerted a lower capacity of migration toward CCL2 in vitro (Fig. 4C). Compared with PCA treatment, Cy-3-G treatment exhibited a higher ability to inhibit CCR2 expression, and to slow the migration of PBMs.

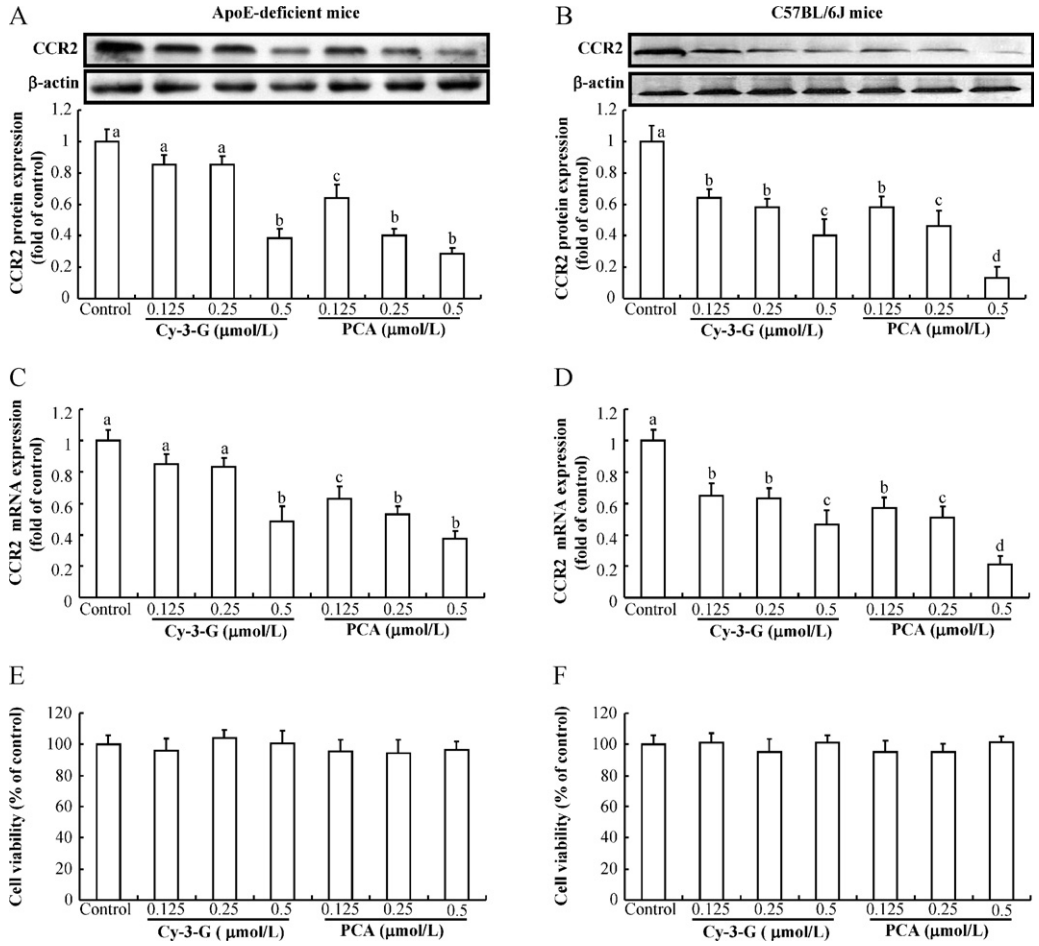

Fig. 2. Effects of Cy-3-G and PCA on CCR2 expression in mouse PBMs. PBMs from the ApoE-deficient and C57BL/6J mice were incubated with indicated concentrations of Cy-3-G or PCA for 24 h. CCR2 expression in PBMs from the ApoE-deficient and C57BL/6J mice was then assessed by Western blotting (A, B) and real time RT-PCR (C, D), respectively. The viability of mouse PBMs from ApoE-deficient (E) and C57BL/6J mice (F) was assessed by MTT assay. Top panel A and B is a representative blot image for CCR2 and β-actin. Bottom panels A and B, protein levels were quantified as described in Section 2. For panel C through F, and bottom panel A and B, results are means ± SEM (n = 6 per group). Values without a common letter differ, $P < 0.05$.

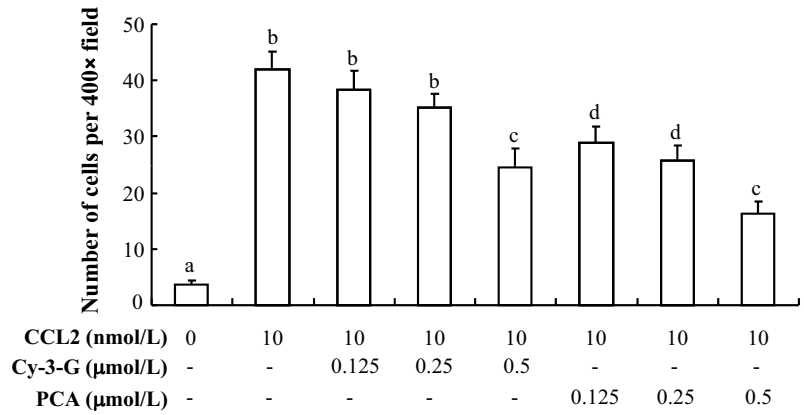

Fig. 3. Effects of Cy-3-G and PCA on the mouse PBMs chemotaxis toward CCL2. PBMs from the ApoE-deficient mice were treated with indicated concentrations of Cy-3-G, PCA, or vehicle DMSO for 24 h. The mouse PBMs were then added to the upper wells of a Boyden chamber, with the lower wells containing CCL2 (10 nmol/L) or its vehicle normal saline. Following incubation at 37 °C for 4 h, cells remaining on the upper surface of the filter were removed out. Migrated cells were counted under the microscope by double blinded investigators. Results are means ± SEM (n = 3 per group). Values without a common letter differ, $P < 0.05$.

3.5. Oral administration of Cy-3-G or PCA reduced macrophage infiltration into the abdominal cavity of ApoE-deficient mice

To investigate whether down-regulation of CCR2 expression by Cy-3-G or PCA treatment could decrease the monocyte chemotaxis in vivo, we used the model of thioglycollate-induced macrophage infiltration into the peritoneal cavity [20,21]. Compared with the control group, oral administration of Cy-3-G (25 mg/kg bw) or PCA (25 mg/kg bw) for one week significantly reduced the macrophage infiltration into the abdominal cavity of the ApoE-deficient mice (Table 1), whereas either Cy-3-G or PCA treatment did not affect the granulocyte and lymphocyte infiltration. During the period of the thioglycollate-induced macrophage infiltration assay, the mice still received Cy-3-G or PCA treatment. Compared with PCA treatment, Cy-3-G treatment displayed a higher capacity of inhibiting the macrophage infiltration into the abdominal cavity.

4. Discussion

It is generally known that polyphenols including anthocyanins are beneficial to the atherosclerotic cardiovascular diseases in populations and animals [2]. Due to the poor understanding of the bioavailability of polyphenols [22], the antiatherogenic mechanism of polyphenols remains largely unknown. Most, if not all, polyphenols after ingestion are rapidly biotransformed into these metabolites, indicating that either parent polyphenols or these metabolites or both are contributed to these protective effects on atherosclerosis. For example, anthocyanins have been shown to be transformed into three main types of metabolites: methylation, sulfation, and glucuronidation in humans [23]. However, a larger number of studies have indicated that the above metabolites are less active than these parent anthocyanins in biological effects [23,24]. Interesting, a human study has identified that PCA was a metabolite of Cy-3-G [14]. Indeed, previous studies have demonstrated that PCA has marked antioxidant/antiinflammatory activities [25,26], and could directly inhibit atherosclerosis development in the ApoE-deficient mouse model [27]. This made us hypothesize that the PCA could contribute to the recognized antiatherogenic effect of its parent Cy-3-G. Nevertheless, it is unclear whether Cy-3-G could be transformed into PCA in the ApoE-deficient mice, thus leading to assist in the inhibitory effect of Cy-3-G on atherosclerosis development.

In the present study, we report for the first time that after orally gavaged with Cy-3-G, Cy-3-G was extensively transformed into PCA, and the plasma maximal concentration of PCA was 3.7-fold higher than that of Cy-3-G in the ApoE-deficient mice. Since the absorbed proportion of Cy-3-G is usually less than 0.1% of oral intake [12], the majority of unabsorbed Cy-3-G subsequently enters into the colon [28]. It is well known that gut microflora plays a critical role in degrading polyphenols [29]. The metabolism of isoflavone into equol by gut microflora is a faithful example [30]. Thus, we speculated that Cy-3-G is metabolized into PCA by gut microflora. Indeed, we evidenced that the metabolite of Cy-3-G, PCA, reached a culmination delaying 1.5 h compared to the PCA group. The lag time may be explained by that Cy-3-G can be metabolized by gut microflora. Furthermore, supporting our speculation, an in vitro study demonstrated that incubation of Cy-3-G with the feces obtained from rats could produce a large quantity of PCA [31].

The infiltration of circulating monocytes into the vascular wall is a typical character of the early stage of atherosclerosis [32]. A number of chemokines and their receptors have involved in this process [33]. Among them, CCL2 and CCR2 play a non-redundant role in the macrophage infiltration and the development of atherosclerosis. Monocytes from CCR2-deficient mice did not migrate toward CCL2. CCR2-deficient mice fed a western diet displayed a smaller aortic sinus lesions and a less number of macrophages infiltration in these lesions compared to wild-type controls [34]. In the current study, we demonstrated that Cy-3-G or PCA at the physiological order of concentrations reduced CCR2 expression in PBMs from both ApoE-deficient and C57BL/6J mice. Furthermore, Cy-3-G or PCA reduced the response of PBMs from

Table 1
Effects of Cy-3-G and PCA treatment on thioglycollate-induced macrophage infiltration into the peritoneal cavity.

Groups	Total cells (×10,000 cells)	Granulocytes (×10,000 cells)	Macrophages (×10,000 cells)	Lymphocytes (×10,000 cells)
Control	2979 ± 191^{a}	432 ± 47^{a}	2532 ± 163^{a}	15 ± 6^{a}
Cy-3-G	1423 ± 109^{b}	419 ± 16^{a}	977 ± 92^{b}	27 ± 9^{a}
PCA	1765 ± 121^{c}	390 ± 17^{a}	1354 ± 103^{c}	21 ± 7^{a}

Note: Values are means ± SEM (n = 12 per group). Values in the same column with different letters are significantly different at $P < 0.05$.

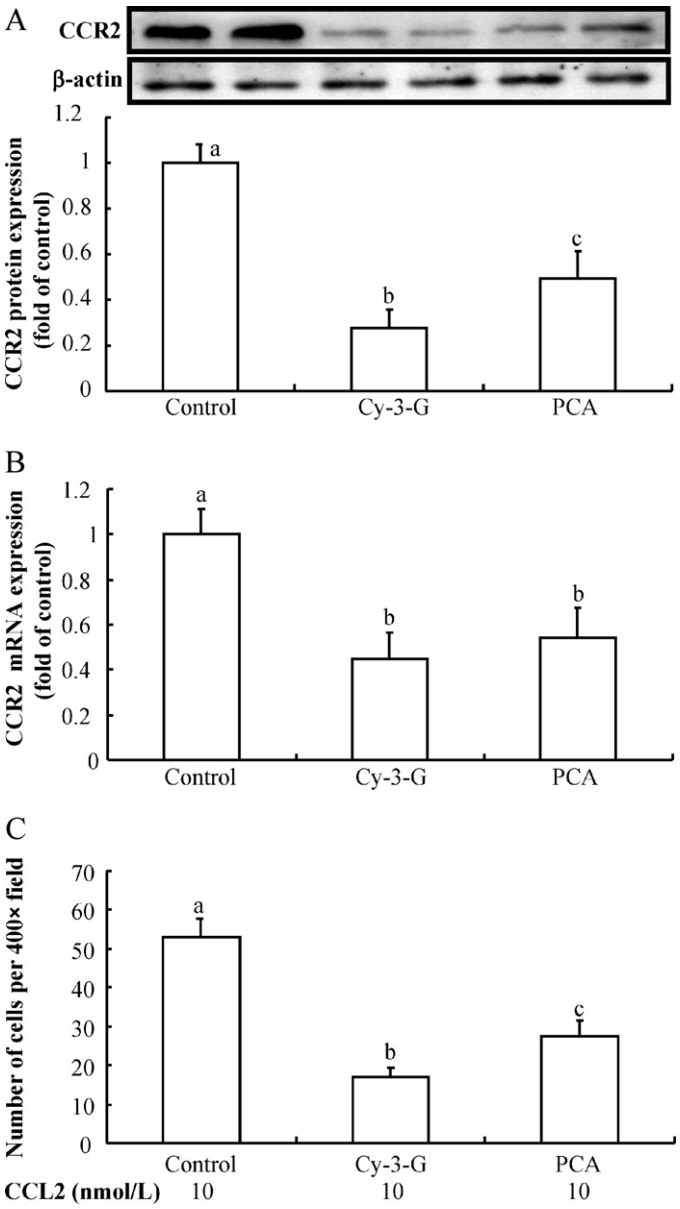

Fig. 4. Oral administration of Cy-3-G or PCA reduced CCR2 expression in mouse PBMs. ApoE-deficient mice were once orally gavaged with Cy-3-G (25 mg/kg bw) or PCA (25 mg/kg bw), or its vehicle normal saline. After 8 h of gavage, the mouse PBMs were isolated and the whole cell lysates were prepared for assessing CCR2 expression by Western blotting (A) and real time RT-PCR (B). Top panel A is a representative blot image for CCR2 and β-actin. Bottom panel A, protein levels were quantified as described in Section 2. For bottom panel A and panel B, results are means ± SEM (n = 6 per group). Values without a common letter differ, $P < 0.05$. C. The isolated mouse PBMs were added to the upper wells of a Boyden chamber, with the lower wells containing CCL2 (10 nmol/L). After incubation at 37 °C for 4 h, migrated cells were counted under the microscope by double blinded investigators. Results are means ± SEM in the observational field (n = 3 per group). Values without a common letter differ, $P < 0.05$.

the ApoE-deficient mice toward CCL2 in a Boyden chamber. Consistently with the in vitro findings, orally administrated Cy-3-G or PCA reduced macrophage infiltration into the abdominal cavity of ApoE-deficient mice. The present study therefore sheds a new light into the potential antiatherogenic properties of anthocyanins in additional to the antioxidative [35] and anti-inflammatory [36] functions. It should be pointed out that between the tested Cy-3-G and PCA, PCA possessed a higher ability to inhibit monocyte migration in vitro. Of interest, the controversial results were observed in mice in the condition of the plasma concentration of Cy-3-G plus PCA was far lower than that of PCA, implicating that there could be a synergetic effect of PCA with Cy-3-G. However, the mechanism by which Cy-3-G and PCA synergetically inhibited macrophage infiltration needs further investigation.

To date, the recognized antiatherogenic properties of polyphenols including anthocyanins, such as antioxidation and antiinflammation, are generally extrapolated from in vitro studies using these parent polyphenols [37]. Importantly, the concentrations of polyphenols used in vitro, were usually far higher than that occurs in vivo [38]. Thus, it is generally not valid to unravel the precise antiatherogenic mechanisms using their parent structures at the far higher physiological concentration. The present finding that Cy-3-G, at the physiological order of concentrations reduced the monocyte migration in vitro and in vivo is thus, of particular importance, which may be a real mechanism for anthocyanins in reducing the severity of atherosclerosis.

Since most, if not all, polyphenols can be absorbed from the intestinal tract and transformed into these metabolites in humans, it is not adequate to predict the health effects by considering their native structures. It is likely that the metabolites of polyphenols facilitate the interpretation of observed protective effects on atherosclerosis in humans. For example, equol, a metabolite of isoflavone, has been demonstrated to be a critical factor for the clinical effectiveness of soy isoflavone in cardiovascular health [39]. Observations made in this study that PCA, a metabolite of Cy-3-G inhibits the monocyte infiltration in the ApoE-deficient mice, may be an important contributor to the antiatherogenic effect of Cy-3-G-rich foods or extract. Thus, future research should pay more attention to the biological properties of the metabolites of polyphenols, in addition to polyphenols.

In conclusion, we demonstrated in this study that Cy-3-G could be intensively transformed into PCA in the ApoE-deficient mice. Moreover, we evidenced that either Cy-3-G or PCA treatment reduces monocyte/macrophage infiltration in vitro and in vivo, at least in part, via negatively modulating the expression of CCR2. Thus, we suggest that Cy-3-G or its metabolite PCA may have a potential clinical implication in reducing the severity of atherosclerosis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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