Bioorganic & Medicinal Chemistry Letters 22 (2012) 3710–3712

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Vascular barrier protective effects of eckol and its derivatives

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ARTICLE INFO

Article history:
Received 2 March 2012
Revised 2 April 2012
Accepted 5 April 2012
Available online 12 April 2012

Keywords:
Eckol
Dieckol
Vascular barrier integrity
Endothelium
LPS

ABSTRACT

In this Letter, we first investigated the barrier protective effects of eckol and its derivatives against pro-inflammatory responses in human umbilical vein endothelial cells (HUVECs) and in mice. Data showed that eckol (**1**) and dieckol (**2**) inhibited lipopolysaccharide (LPS)-mediated barrier disruption and trans-endothelial migration of leukocytes to human endothelial cells. Eckol (**1**) also suppressed acetic acid induced-hyperpermeability and carboxymethylcellulose-induced leukocytes migration in vivo. Interestingly, the barrier protective effects of dieckol (**2**) were better than those of eckol (**1**) and hydroxyl groups in dieckol (**2**) positively regulate protective effects.

Vascular endothelial barrier serves to separate the inner space of the blood vessel from the surrounding tissue and to control the exchange of cells and fluids between the two.[1,2] This barrier exhibits a variety of important functions, including control of coagulation, fibrinolysis, vascular tone, growth and immune response.[3] And it is dynamic and highly susceptible to the regulation by various stimuli of physiological and pathological origin.[1,2] It is well recognized that disruption of vascular barrier integrity results in marked increases in permeability to fluid and solute and is necessary to provide the access of leukocytes to the inflamed tissues and the central pathophysiologic mechanism of many vascular inflammatory disease processes such as sepsis and atherosclerosis.[2,4,5] Thus, altered permeability of the endothelial barrier is a characteristic hallmark of inflammatory responses which contributes to the morbidity and mortality in several inflammatory diseases such as sepsis, acute lung injury and anaphylaxis.[6,7] In addition, as an initial event of inflammation, leukocytes adhere to the vascular lining and migrate into the inflamed tissue.[8] The adhesion of circulating leukocytes to the vascular endothelium is a fundamental step in leukocyte extravasation during inflammation.[9,10] In particular, endothelial dysfunction is related to leukocyte recruitment during the formation of the inflammatory lesion.[11,12] Therefore, inhibition of leukocytes migration to vascular endothelium and barrier permeability as a therapeutic approach is an attractive way to potentially prevent early inflammatory injury. Therefore, agents that inhibit the leukocyte migration to vascular endothelium and enhance endothelial cell barrier function are desirable for a variety of inflammatory diseases.[2,13]

The search for anticancer drugs and anti-inflammatory agents from natural products represents an area of great interest worldwide.[14] *Eisenia bicyclis* is a common perennial brown alga of the family Laminariaceae that inhabits the middle Pacific coast around Korea and Japan.[15] This seaweed is consumed as appetisers, casseroles, muffins, pilafs, soups, toasted dishes and many other types of food. The known biological activities of this brown alga[15] include effects on skin disease, Alzheimer's disease, allergies, diabetes and cancer.[15–17] Previously, antioxidant activity of *E. bicyclis* phlorotannins such as eckol (**1**) and dieckol (**2**) has been reported.[15] However, the effect of eckol (**1**) and its derivatives (**2**–**4**) on vascular barrier integrity in both cellular system and animal model have not yet been elucidated (Fig. 1). The objective of the present study was to test naturally occurring anti-inflammatory agent from *E. bicyclis*[16] for its vascular barrier protective effect on endothelial cells and mice.

To determine the effects of purified eckol or dieckol[18] on the HMGB1-mediated hyperpermeability primary HUVECs, the flux of albumin in a dual chamber system was monitored as described previously.[19] The inhibitory effect of dieckol (**2**) was better than that of eckol (**1**) (Table 1). The possible explanation for these results is the number and position of hydrogen donating hydroxyl groups in eckol (**1**) or dieckol (**2**). To verify this hypothesis, the hydroxyl groups of dieckol (**2**) were changed to methyl groups or acetyl groups. To do

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http://dx.doi.org/10.1016/j.bmcl.2012.04.026

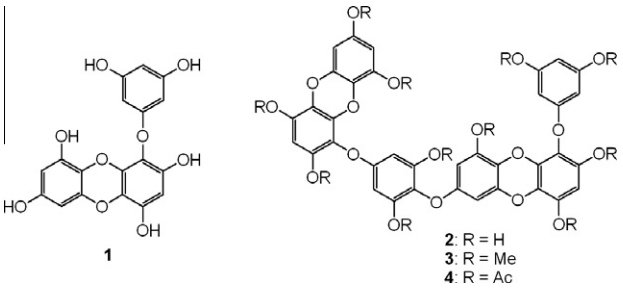


Figure 1. Structures of eckol and its derivatives.

Table 1
Effects of different eckols on LPS-mediated hyperpermeability in HUVECs

Compound	Dose	ELISA OD_{650}	Inhibition (%)
(−) Control (PBS)		0.096 ± 0.013	
(+) Control (LPS)	100 ng/ml	0.554 ± 0.042	
Eckol (**1**)	10 μM	0.242 ± 0.025*	56.5
Dieckol (**2**)	10 μM	0.138 ± 0.014**	74.9
Me-dieckol (**3**)	10 μM	0.371 ± 0.012*	33.0
Ac-dieckol (**4**)	10 μM	0.278 ± 0.028*	49.5
Kaempferol-3-*O*-sophoroside[a]	10 μM	0.129 ± 0.019**	76.6

Each data represent the mean ± S.D. of three different experiments.
* <0.05 significantly different from the LPS.
** <0.01 significantly different from the LPS.
[a] Used as positive control.

Table 2
Effects of different eckols on acetic acid-mediated hyperpermeability in mice

Compound	Dose	μg/mouse (n = 5)	Inhibition (%)
(−) Control (PBS)		0.46 ± 0.07	
(+) Control (acetic acid)	0.7%	5.54 ± 0.42	
Eckol (**1**)	10 μM	2.65 ± 0.09**	52.0
Dieckol (**2**)	10 μM	1.41 ± 0.23**	74.4
Me-dieckol (**3**)	10 μM	3.61 ± 0.08*	34.7
Ac-dieckol (**4**)	10 μM	2.24 ± 0.04**	59.5
Kaempferol-3-*O*-sophoroside[a]	10 μM	1.45 ± 0.18**	73.9

Each data represent the mean ± S.D. of three different experiments.
* <0.05 significantly different from the acetic acid.
** <0.01 significantly different from the acetic acid.
[a] Used as positive control.

Table 3
Effects of different eckols on LPS-mediated monocyte migration on HUVECs.

Compound	Dose	Migration Index	Inhibition (%)
(−) Control (PBS)		27.9 ± 4.7	
(+) Control (LPS)	100 ng/ml	163.6 ± 6.7	
Eckol (**1**)	10 μM	65.9 ± 6.5**	59.6
Dieckol (**2**)	10 μM	46.8 ± 8.4**	71.3
Me-dieckol (**3**)	10 μM	101.9 ± 5.1*	37.6
Ac-dieckol (**4**)	10 μM	81.0 ± 9.1**	50.3
Kaempferol-3-*O*-sophoroside[a]	10 μM	49.8 ± 3.7**	69.5

Each data represent the mean ± S.D. of three different experiments.
* <0.05 significantly different from the LPS.
** <0.01 significantly different from the LPS.
[a] Used as positive control.

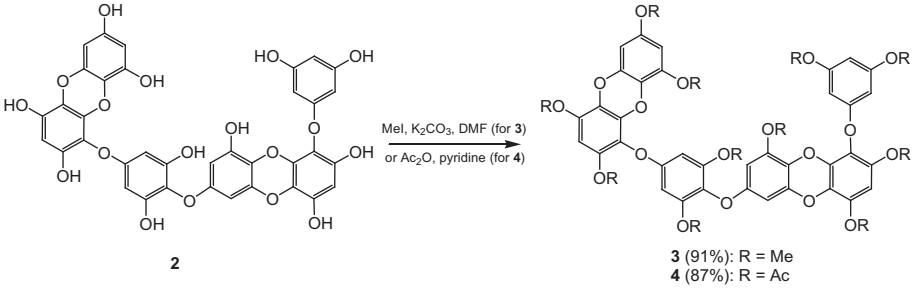
these, the methylation and acetylation reactions of hydroxyl groups in dieckol (**2**) promoted by treatment with iodomethane and acetic anhydride furnished the respective Me-dieckol (**3**) (91%) and Ac-dieckol (**4**) (87%) (Scheme 1).[20]

As shown in Table 1, the inhibitory effect of dieckol was diminished when its hydroxyl groups were changed to methyl groups. However, when the hydroxyl groups of dieckol were changed to acetyl groups, the inhibitory effect of Ac-dieckol (**4**) was better than that of Me-dieckol (**3**). These results suggest that the existence of hydrogen bond donors and hydrophilic moieties are important to inhibitory effects. The Me-dieckol (**3**) and Ac-dieckol (**4**) without the hydroxyl groups as hydrogen bond donors showed the less inhibitory effects than the dieckol (**2**). The inhibitory effect of more hydrophilic acetyl substituted dieckol (**4**) was higher than its less hydrophilic methyl substituted dieckol (**3**) (see Table 1).

To confirm this effect in vivo, acetic acid-induced vascular permeability in mice was assessed as described previously.[21] As shown in Table 2, eckol (**1**–**4**) markedly inhibited the leakage of dye into the peritoneum in mice. To exclude the possibility that the inhibition of permeability was due to cytotoxicity caused by each eckol (**1**–**4**), cellular viability assays were performed in HUVECs treated with each eckol for 24 h. At the concentrations used (up to 20 μM), each eckol (**1**–**4**) did not affect cell viability (data not shown).

The adhesion of leukocytes to endothelial cells and transendothelial migration (TEM) of leukocytes are important steps in the pro-inflammatory response.[22,23] We conducted studies to determine whether eckol could block the adhesion of monocytes to HMGB1-stimulated HUVECs. We demonstrated that eckol effectively inhibited the binding of monocytes to HMGB1-stimulated endothelial cells (data not shown). Further studies revealed that the adhesion of monocytes to endothelial cells was associated with their subsequent TEM and that eckol also effectively inhibited this step (Table 3). To confirm this effect in vivo, CMC–Na-induced leukocyte migration in mice was examined. CMC–Na significantly stimulated leukocyte migration into the peritoneal cavity of mice and eckol at doses of 10 μM significantly decreased leukocytes counts (Table 4). Kaempferol-3-*O*-sophoroside was used as a positive control in biological test.[24]

Collectively, above data showed that these barrier protective effects of dieckol (**2**) were better than eckol (**1**). Recent studies



Scheme 1. Synthesis of dieckol derivatives **3** and **4**.

Table 4
Effects of different eckols on CMC–Na-mediated leukocytes migration in mice.

Compound	Dose	$\times 10^6$	Inhibition (%)
(−) Control (PBS)		1.20 ± 0.15	
(+) Control (CMC–Na)	1.0%	6.00 ± 0.29	
Eckol (**1**)	10 μM	2.30 ± 0.28**	61.7
Dieckol (**2**)	10 μM	1.80 ± 0.14**	69.9
Me-dieckol (**3**)	10 μM	4.05 ± 0.07*	32.4
Ac-dieckol (**4**)	10 μM	2.60 ± 0.14**	56.7
Kaempferol-3-*O*-sophoroside[a]	10 μM	1.81 ± 0.23*	69.6

Each data represent the mean ± S.D. of three different experiments.
* <0.05 significantly different from the CMC–Na.
** <0.01 significantly different from the CMC–Na.
[a] Used as positive control.

have demonstrated that the protective effect against oxidative stress induced by ROS and UV radiation is correlated with the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phonolic molecules, and is also affected by other factors, such as other H-donating groups (–NH, –SH), etc.[25,26] Our results indicated that dieckol (**2**) has more functional hydroxyl groups than other tested eckol, therefore, this study with dieckol unravels a novel vascular barrier protective functions.

Acknowledgment

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government [MEST] (Nos. 2011-0026695, 2011-0030124).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.026.

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18. Fresh *E. bicyclis* was washed three times with water to remove salt. Lyophilized *E. bicyclis* was ground into powder before extraction. The dried *E. bicyclis* powder (1.0 kg) was extracted with MeOH (10 L × 3) at room temperature and the solvent was evaporated in vacuo. The combined crude MeOH extract (164.3 g) was suspended in 10% MeOH (1.0 L), and then partitioned in turn with *n*-hexane (1.0 L × 3), CH_2Cl_2 (1.0 L × 3), EtOAc (1.0 L × 3), and *n*-BuOH (1.0 L × 3) to yield dried *n*-hexane- (42.3 g), CH_2Cl_2 (2.5 g), EtOAc- (23.0 g), *n*-BuOH (26.5 g) and H_2O-soluble (69.1 g) residues. A portion (10.0 g) of the EtOAc extract was chromatographed on a Sephadex LH-20 column (4.0 cm i.d. × 50 cm) with MeOH and fractioned into seven subfractions (EB01–EB07). Subfractions EB02 and EB07 were subjected to column chromatography over a LiChroprep RP-18 column (1.1 cm i.d. × 37 cm) with aqueous MeOH to yield pure eckol (**1**) (t_R 4.0 min, 25.2 mg) and dieckol (**2**) (t_R 8.1 min, 17.2 mg).
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20. *Preparation of Me-dieckol (**3**).* To a solution of dieckol (**2**, 45 mg, 0.061 mmol) in DMF (5 mL) were added iodomethane (0.10 mL, 1.60 mmol) and potassium carbonate (276 mg, 2.00 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 9 h, and then diluted with EtOAc, washed with 1 N HCl and brine, dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash silica gel column chromatography (hexane/EtOAc, 1:1) to give 7-[2,6-dimethoxy-4-(2,4,7,9-tetramethoxydibenzo[*b*,*e*][1,4]dioxin-1-yloxy)phenoxy]-1-(3,5-dimethoxyphenoxy)-2,4,9-trimethoxydibenzo[*b*,*e*][1,4]dioxine (**3**, 50 mg, 91%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.70 (s, 6H), 3.72 (s, 6H), 3.74 (s, 6H), 3.75 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.94 (s, 3H), 5.94 (d, *J* = 3.0 Hz, 1H), 6.11–6.16 (m, 4H), 6.20–6.22 (m, 2H), 6.30 (s, 1H), 6.32 (s, 2H), 6.34 (d, *J* = 2.7 Hz, 1H); LC–MS (ESI) *m*/*z* 897 ([M+1]$^+$).
*Preparation of Ac-dieckol (**4**).* To a solution of dieckol (**2**, 18 mg, 0.024 mmol) in pyridine (4 mL) was added acetic anhydride (0.050 mL, 0.53 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 6 h, and then diluted with EtOAc, washed with saturated $CuSO_4$, H_2O, and brine, dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash silica gel column chromatography (hexane/EtOAc, 1:1) to give 4-{3,5-diacetoxy-4-[4,7,9-triacetoxy-6-(3,5-diacetoxyphenoxy)dibenzo[*b*,*e*][1,4]dioxin-2-yloxy]phenoxy}dibenzo[*b*,*e*][1,4]dioxine-1,3,6,8-tetrayl tetraacetate (**4**, 25 mg, 87%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93 (s, 3H), 2.04 (s, 3H), 2.11 (s, 6H), 2.13 (s, 3H), 2.19 (s, 3H), 2.245 (s, 3H), 2.254 (s, 6H), 2.28 (s, 3H), 2.34 (s, 3H), 6.31 (d, *J* = 2.7 Hz, 1H), 6.43 (d, *J* = 3.0 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.57 (d, *J* = 2.1 Hz, 2H), 6.62 (s, 1H), 6.63 (d, *J* = 3.0 Hz, 1H), 6.67 (s, 1H), 6.68 (s, 2H), 6.70 (t, *J* = 2.0 Hz, 1H).
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