JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 1315–1321

Received April 7, 2004
Accepted June 21, 2004
DOI 10.1211/0022357044229
ISSN 0022-3573

Peroxynitrite scavenging mode of alaternin isolated from *Cassia tora*

Tae Hyun Park, Dae Hyun Kim, Chul Hong Kim, Hyun Ah Jung, Jae Sue Choi, Jae Won Lee and Hae Young Chung

Abstract

Peroxynitrite ($ONOO^-$), formed from the reaction of superoxide ($\cdot O_2^-$) and nitric oxide ($\cdot NO$), is a potent oxidant that contributes to the oxidation of various cellular constituents, including lipids, amino acids, sulfhydryls and nucleotides. It can cause cellular injury, such as DNA fragmentation and apoptotic cell death. $ONOO^-$ toxicity is also reported to be involved in inflammatory and neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and atherosclerosis. Moreover, the necessity for a strong $ONOO^-$ scavenger is important because of the lack of endogenous enzymes that protect against the damage caused by $ONOO^-$. The aim of this study was to evaluate the ability of natural products to scavenge $ONOO^-$. We tested various plant extracts for their $ONOO^-$ scavenging activity. Among them, extract from *Cassia tora*, which is well known as an oriental herb in traditional medicine, showed potent $ONOO^-$ scavenging activity. Further analysis identified the phenolic active components, alaternin and nor-rubrofusarin glucose, as potent $ONOO^-$ scavengers. Spectrophotometric analysis demonstrated that alaternin and nor-rubrofusarin glucose led to a decrease in the $ONOO^-$-mediated nitration of tyrosine through electron donation. In bovine serum albumin, alaternin, but not nor-rubrofusarin glucose, showed significant inhibition of $ONOO^-$-mediated nitration in a dose-dependent manner. We believe alaternin can be developed as an effective $ONOO^-$ scavenger for the prevention of $ONOO^-$-associated diseases.

Introduction

The reactive species peroxynitrite ($ONOO^-$) is derived from the reaction between nitric oxide ($\cdot NO$) and superoxide anion ($\cdot O_2^-$) (Beckman et al 1990) and is a potent cytotoxicant that contributes to carcinogenesis and to aging. When stimulated, immune cells, such as macrophages, endothelial cells and kupffer cells, release $\cdot NO$ and $\cdot O_2^-$ (Huie & Padmaja 1993). At physiological pH ($pK_a = -6.8$) $ONOO^-$ is protonated to form peroxynitrous acid, which decays rapidly to form a mixture of highly toxic oxidizing and nitrating species, apparently including the hydroxyl radical ($\cdot OH$) (Beckman et al 1994; Van der Vliet et al 1994). $ONOO^-$ can induce lipid peroxidation, protein nitration and oxidation of thiol (-SH) groups (Stepien et al 2000). Furthermore, $ONOO^-$ directly inhibits the activity of mitochondrial respiratory chain enzymes and changes membrane potential and permeability. It can also cause cellular injury with DNA fragmentation and apoptotic cell death (Darley-Usmar & Halliwell 1996; Spencer et al 1996). Recently, toxicity by $ONOO^-$ has been reported in inflammatory and neurodegenerative diseases such as atherosclerosis, Alzheimer's disease, and Parkinson's disease (Kaur & Halliwell 1994; Darley-Usmar et al 1995; Kooy et al 1995). Moreover, the necessity for a strong $ONOO^-$ scavenger is important because of the lack of endogenous enzymes that protect against the damage caused by $ONOO^-$.

Many previous studies report on various $ONOO^-$ scavengers, such as selenocystine, selenomethionine and ebselen (Sies & Masumoto 1997), melatonin (Cuzzocrea et al 1999), ascorbic acid (Sandoval et al 1997), α-tocopherol and flavonoids (Haenen et al 1997), isoflavonoids (Boersma et al 1999), ergothioneine (Hartman 1990; Aruoma et al 1997) and polyhydroxyphenols (Chung et al 1998a).

College of Pharmacy, Aging Tissue Bank, Pusan National University, Busan 609-735, Korea

Tae Hyun Park, Dae Hyun Kim, Chul Hong Kim, Jae Won Lee, Hae Young Chung

Faculty of Food Science and Biotechnology, Pukyong National University, Pusan 608-737, Korea

Hyun Ah Jung, Jae Sue Choi

Correspondence: H. Y. Chung, College of Pharmacy, Pusan National University, San 30, Tangjun-dong, Gumjung-gu, Busan, 609-735, Korea.
E-mail: hyjung@pusan.ac.kr

Funding: This work was supported by Grant R01-2000-000-00120-0 from the Basic Research Program of the Korea Science and Engineering Foundation.

Cassia tora is a shrub that is well known in traditional medicine among the people of Asia. Its extracts show antimutagenic properties (Choi et al 1997) and radical scavenging effects (Choi et al 1994). Antioxidant properties (Yen & Chuang 2000), as well as inhibitory effects against DNA damage (Wu et al 2001), of water extracts of *Cassia tora* are also reported. Furthermore, compounds of *Cassia tora* are shown to have antibacterial effects (Hatano et al 1999).

However, there is no previous report on the effect of components of *Cassia tora* on $ONOO^-$. Therefore, we examined the $ONOO^-$ scavenging activity of two active *Cassia tora* compounds, alaternin and nor-rubrofusarin glucose, against the biological damage caused by $ONOO^-$.

Materials and Methods

Materials

DL-Penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St Louis, MO). Dihydrorhodamine-123 (DHR 123) and $ONOO^-$ were from Molecular Probe (Eugene, OR) and Cayman Chemical Co. (Ann Arbor, MI), respectively. 4,5-Diaminofluorescein (DAF-2) was purchased from Daiichi Pure Chemicals Co. (Tokyo, Japan). Poly (vinylidene fluoride) membrane (Immobilon-P) was obtained from Millipore Corp. (Bedford, MA) and the chemiluminescence detection system was from Amersham Life Sciences Inc. (Arlington Heights, IL). Anti-nitrotyrosine antibody and horseradish peroxidase-conjugated anti-mouse secondary antibody from sheep were obtained from Upstate Biotechnology (Lake Placid, NY) and Amersham (Piscataway, NJ), respectively. All other chemicals were of the highest purity available from either Sigma Chemical Co (St Louis, MO) or Junsei Chemical Co. (Tokyo, Japan).

Isolation of alaternin and nor-rubrofusarin glucose

Alaternin and nor-rubrofusarin glucose were isolated from *C. tora L.* as described previously (Choi et al 1994). The powdered dried seeds of *C. tora L.* were extracted by a reflux process for 4 h using methyl alcohol (MeOH). The filtrates were concentrated to dryness in a vacuum at 95°C to yield the MeOH extract. The MeOH extract was chromatographed over a silica gel column and eluted with CH_2Cl_2–EtOAc and n-BuOH to obtain different fractions. The CH_2Cl_2 layer fraction was subjected to column chromatography over a silica gel column with CH_2Cl_2–MeOH (gradient) to give alaternin. Nor-rubrofusarin was obtained from the combined CH_2Cl_2 layer and EtOAc fraction. The detailed information on isolation of other components were reported previously (Choi et al 1994). All components were identified by comparison with the authentic sample purchased from Sigma. The chemical structures of alaternin and nor-rubrofusarin glucose are illustrated in Figure 1.

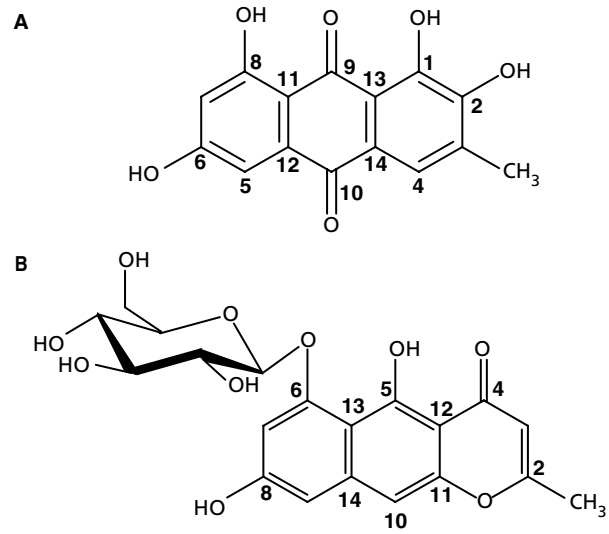

Figure 1 Structures of alaternin (A) and nor-rubrofusarin glucose (B).

Measurement of $ONOO^-$ scavenging activity

All compounds were dissolved in 10% EtOH for $ONOO^-$ and other free radical scavenging assays. The concentration range was from 200 μM to 8 μM and three end-points were detected. $ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123 by a modified method of Kooy et al (1994). A stock solution of 5 mM DHR 123 in dimethylformamide was purged with nitrogen and stored at −20°C. A working solution of 5 μM DHR 123 diluted from the stock solution was placed on ice in the dark immediately before the study. The buffer (composition in mM: 90 sodium chloride, 50 sodium phosphate (pH 7.4) and 5 potassium chloride) was purged with nitrogen and placed on ice before use. Just before use, 100 μM diethylenetriaminepenta acetic acid (DTPA) was added. Then the buffer mixture was added to the solutions of compounds, which were loaded in 96-well plates. $ONOO^-$ scavenging by the oxidation of DHR 123 was measured using a microplate fluorescence spectrophotometer FL 500 (Bio-Tek Instruments, Winooski, VT) with excitation and emission wavelengths of 485 nm and 530 nm, respectively, at room temperature. The background and final fluorescent intensities were measured 5 min after treatment with or without authentic 10 μM $ONOO^-$ in 0.3 M NaOH. Authentic $ONOO^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time. The $ONOO^-$ solution was quantified spectrophotometrically ($OD_{302} = 1670\ M^{-1}\,cm^{-1}$) using the Ultraspec 2000 UV/visible spectrophotometer (Pharmacia-Biotech, Oxford, England). The changes in fluorescence between control (10% EtOH) and samples were recognized as $ONOO^-$ scavenging activity.

The IC50 value was calculated by the following method. First, the scavenging activity curve of each compound was obtained through 3 independent experiments of $ONOO^-$ scavenging by the compound in various doses. Then IC50 was determined as the average of three doses in which 50% $ONOO^-$ was scavenged.

Measurement of $\cdot O_2^-$ scavenging activity

2,7-Dichlorodihydrofluorescein diacetate (H_2DCFDA) was oxidized to fluorescent 2,7-dichlorofluorescein (DCF) by reactive species (Cathcart et al 1983, 1984; LeBel et al 1992). A stock solution of 12.5 mM H_2DCFDA in 100% EtOH was stored at −20°C. A working solution of 25 μM H_2DCFDA diluted from the stock solution was placed on ice in the dark immediately before the study. The fluorescence intensity of DCF was measured by using a microplate fluorescence spectrophotometer (FL 500; Bio-Tek Instruments) with excitation and emission wavelengths of 485 nm and 530 nm, respectively, for 1 h with or without the addition of menadione (20 mM) as a source of reactive species.

Measurement of ·NO scavenging activity

·NO scavenging was measured by monitoring 4,5-diaminofluorescein (DAF-2) using a modified method of Chung et al (2001). DAF-2, as a specific ·NO indicator, selectively traps ·NO between two amino groups in its molecule and yields triazolofluorescein, which emits green fluorescence when excited at 490–495 nm (Nagata et al 1999). A stock solution of 1 mg DAF-2 in 0.55 mL dimethyl sulfoxide was stored at −20°C. A working solution of 0.5 μg DAF-2 was diluted with 50 mM phosphate buffer (pH 7.4) purged with nitrogen. An ·NO donor, 2 mM sodium nitroprusside, was added to a 96-well microplate. The fluorescence intensity was dependent on the amount of ·NO trapped by DAF-2. The fluorescence signal caused by the reaction of DAF-2 with ·NO was measured using a fluorescence spectrometer (FL 500; Bio-Tek Instruments) at excitation and emission wavelengths of 485 nm and 530 nm after 10 min.

Reaction of alaternin and nor-rubrofusarin glucose with $ONOO^-$

The reaction between alaternin and nor-rubrofusarin glucose and $ONOO^-$ was investigated. $ONOO^-$ (500 μM) was reacted with alaternin and nor-rubrofusarin glucose (50 μM) in 50 mM phosphate buffer at pH 7.0 at room temperature for 10 min. Following the reaction, we performed a spectrophotometric scan using a UV/visible spectrophotometer (Ultrospec 2000; Pharmacia Biotech), with wavelengths from 190 nm to 600 nm.

Inhibition of $ONOO^-$-mediated tyrosine nitration by alaternin and nor-rubrofusarin glucose

Utilizing spectrophotometric analysis and Western blot analysis, the ability of alaternin and nor-rubrofusarin glucose to inhibit the formation of 3-nitrotyrosine was quantitated as the index of alaternin and nor-rubrofusarin glucose inhibition of tyrosine nitration.

For spectrophotometric analysis of nitrated proteins, tyrosine (400 μM) was reacted with $ONOO^-$ (500 μM) in the presence of varying concentrations of alaternin and nor-rubrofusarin glucose (0–100 μM). The formation of 3-nitrotyrosine was determined by UV/visible spectrophotometer. Tyrosine was monitored at 275 nm, while 3-nitrotyrosine formation was monitored at 430 nm. The disappearance of 3-nitrotyrosine peaks at 430 nm in the presence of alaternin and nor-rubrofusarin glucose was taken as an indication of the inhibition by alaternin and nor-rubrofusarin glucose.

For Western blotting analysis, 2.5-mL samples of alaternin and nor-rubrofusarin glucose dissolved in 10% v/v EtOH were added to 95 μL of BSA (0.5 mg protein/mL). The mixed samples were incubated with shaking at 20°C for 1 h. After the 1-h incubation, 2.5 μL of $ONOO^-$ (100 μM) in 0.3 M NaOH was added. The samples were then incubated for 30 min at 20°C with shaking.

The prepared samples in a gel loading buffer (pH 6.8; 0.125 M Tris (hydroxymethyl) aminomethane, 4% m/v sodium dodecyl sulfate (SDS), 20% m/v glycerol, 10% m/v 2-mercaptoethanol and 0.2% m/v bromophenol blue) at a ratio of 1:1 were boiled for 5 min. Twenty microlitres of each sample were separated on an SDS-polyacrylamide minigel (10% for BSA) at 100 V and was transferred to a poly (vinylidene fluoride) membrane at 100 V for 1.5 h in a wet transfer system (Bio-Rad, Hercules, CA).

The membrane was immediately placed into a blocking solution (10% m/v skimmed milk powder in TBS–Tween buffer containing 10 mM Tris, 100 mM NaCl and 0.1 mM Tween-20, pH 7.5) at 4°C overnight. The membrane was washed in TBS–Tween buffer for 30 min and then incubated with a monoclonal anti-nitrotyrosine antibody (0.5% m/v skimmed milk, diluted 1:2000 in TBS–Tween buffer) at room temperature for 2 h. After 4 × 10-min washings in TBS–Tween buffer, the membrane was reacted with a horseradish peroxidase-conjugated anti-mouse secondary antibody from sheep (0.1% m/v skimmed milk diluted 1:2000 in TBS–Tween buffer) at room temperature for 2 h. After 4 × 10-min washings in TBS–Tween buffer, antibody labelling was detected using enhanced chemiluminescence and exposed to radiographic film. Pre-stained blue protein marker was used for molecular weight determination.

Statistical analysis

All results are expressed as mean ± s.d. of three separate experiments unless stated otherwise. Statistical analysis was determined by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

$ONOO^-$ scavenging activity of alaternin and nor-rubrofusarin glucose isolated from *Cassia tora*

Alaternin, penicillamine, toralactone gentiobioside, rubrofusarin gentiobioside, adenosine, isofubrofusarin gentiobioside and nor-rubrofusarin glucose from *Cassia tora* were identified. Then a 50% inhibition concentration (IC50) was calculated. Tables 1 and 2 present the IC50 values of these active components for $ONOO^-$, ·NO and $\cdot O_2^-$ scavenging activity. Compared with penicillamine (0.97 ± 0.16 μM),

Table 1 Peroxynitrite scavenging activity of compounds isolated from *Cassia tora*

Compounds of *Cassia tora*	IC50 (μM)
Nor-rubrofusarin glucose	1.78 ± 0.16
Alaternin	2.70 ± 1.07
Toralactone gentiobioside	6.06 ± 1.40
Rubrofusarin gentiobioside	8.65 ± 2.35
Adenosine	$\gg 20$
Isofubrofusarin gentiobioside	$\gg 20$
Penicillamine[a]	0.97 ± 0.16

[a]Used as a positive control. IC50 is the 50% inhibition concentration.

Table 3 Interactions of alaternin and nor-rubrofusarin glucose with peroxynitrite

	A	B	C	D
Alaterin (50 μM)	+	+	−	−
Nor-rubrofusarin glucose (50 μM)	−	−	+	+
$ONOO^-$ (500 μM)	−	+	−	+
OD 430 nm Absorbance	0.02	0.05	0.15	0.08

Each mixed solution was incubated at 37°C with shaking for 1 h and scanned between 190 and 600 nm using spectrophotometric analysis.

Table 2 IC50 (μM) of alaternin and nor-rubrofusarin glucose on related free radicals scavenging activity

Components	·NO	$\cdot O_2^-$
Alaternin	3.99 ± 0.06	17.98 ± 1.60
Nor-rubrofusarin glucose	17.57 ± 0.81	12.24 ± 5.34
Carboxy-PTIO[a]	16.73 ± 2.28	
Trolox[a]		7.24 ± 0.25

[a]Used as a positive control. Carboxy-PTIO was used as ·NO scavenging activity positive control and Trolox was used as $\cdot O_2^-$ scavenging activity positive control. ·NO, nitro oxide radical; $\cdot O_2^-$, superoxide.

Table 4 Effects of alaternin and nor-rubrofusarin glucose on peroxynitrite mediated 3-nitrotyrosine

Tyrosine (400 μM)	+	−	+	+
$ONOO^-$ (500 μM)	+	+	+	+
Alaterin (50 μM)	−	−	+	−
Nor-rubrofusarin glucose (50 μM)	−	−	−	+
OD 430 nm Absorbance	0.35	0	0.15	0.21

Each mixed solution was incubated at 37°C with shaking for 1 h and scanned between 190 and 600 nm using spectrophotometric analysis. The spectrum of the peak displayed at 430 nm reflects the formation of 3-nitrotyrosine.

alaternin (2.70 ± 1.07 μM) and nor-rubrofusarin glucose (1.78 ± 0.16 μM) showed especially potent $ONOO^-$ scavenging activity. Table 2 shows the results of our experiments with alaternin and nor-rubrofusarin glucose. These components (alaternin, 17.98 ± 1.60 μM; nor-rubrofusarin glucose, 12.24 ± 5.34 μM) were shown to scavenge $\cdot O_2^-$ effectively compared with positive control, trolox (7.24 ± 0.25 μM). Alaternin and nor-rubrofusarin glucose also showed strong ·NO scavenging activity with IC50s of 3.99 ± 0.06 and 17.57 ± 0.81 μM, respectively, compared with positive control, carboxy-PTIO (16.73 ± 2.28 μM).

Reaction of alaternin and nor-rubrofusarin glucose with $ONOO^-$

To establish whether alaternin or nor-rubrofusarin glucose undergoes a nitration reaction after the addition of $ONOO^-$, a spectrophotometric analysis was performed to reveal any changes in absorbance at 430 nm (Pannala et al 1998). The interaction of neither alaternin nor nor-rubrofusarin glucose with $ONOO^-$ produced spectral changes at 430 nm as compared with the original spectrum (Table 3), indicating no nitration formation. These results indicate that alaternin and nor-rubrofusarin glucose interact with $ONOO^-$ via electron donation, but not nitration, and this may be regarded as the neutralizing mechanism.

Effect of alaternin and nor-rubrofusarin glucose on $ONOO^-$-mediated 3-nitrotyrosine formation

A peak reaction of tyrosine with $ONOO^-$ was observed at 430 nm, resulting in 3-nitrotyrosine (Table 4). The nitration of tyrosine was easily detectable by the colour change from colourless to the characteristic yellow when tyrosine and $ONOO^-$ were mixed. Once nitrotyrosine was formed at pH 7.4, absorbance was maximal in the 420–440 nm range. Incubation of alaternin and nor-rubrofusarin glucose with tyrosine before the addition of $ONOO^-$ resulted in a reduction in the size of the nitrotyrosine peak at 430 nm, implying that both alaternin and nor-rubrofusarin glucose inhibited the formation of 3-nitrotyrosine.

Effect of alaternin and nor-rubrofusarin glucose on nitration of BSA

A major product resulting from the reaction of proteins with $ONOO^-$ is nitrotyrosine (Ischiropoulos et al 1995). Monoclonal antibodies specific for nitrotyrosine were used to detect nitrotyrosine in BSA (0.5 mg protein/mL) after treatment with $ONOO^-$. When pre-incubated with alaternin or nor-rubrofusarin glucose at concentrations of 20, 40 and 80 μM, we observed that alaternin (Figure 2A), but not nor-rubrofusarin glucose (Figure 2B), attenuated

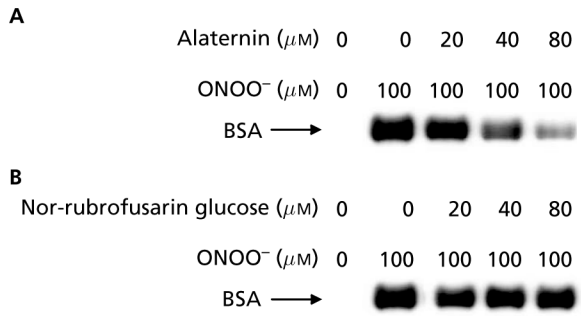

Figure 2 Effect of alaternin (A) and nor-rubrofusarin glucose (B) on the nitration of bovine serum albumin (BSA) by peroxynitrite ($ONOO^-$). Alaternin and nor-rubrofusarin glucose were added to BSA. The reaction samples were incubated with shaking at 20°C for 1 h. After $ONOO^-$ was added, all samples were further incubated with shaking at 20°C for 30 min.

the nitration of BSA in a dose-dependent manner. These results indicate that alaternin may protect significantly against the oxidative damage to BSA.

Discussion

$ONOO^-$ is a mediator of toxicity in inflammatory processes and atherogenesis, having strong oxidizing properties in biological molecules (Sies et al 1998). The toxicity of $ONOO^-$ can be attributed to the nitration of tyrosine and tryptophan residues and to protein function alterations (Elliott et al 1998). Plant-food-derived antioxidants and active principles, such as flavonoids, hydroxycinnamates (ferulic acid, chlorogenic acids, vanilin, etc.), beta-carotene, other carotenoids, vitamin E and vitamin C are increasingly proposed as important dietary antioxidant substances (Aruoma 1999). Many plant foods contain tannins (commonly referred to as tannic acid), which are water-soluble polyphenols and have components that are reported to be anti-carcinogenic (Chung et al 1998b).

Our experiments showed that two isolated active components from *Cassia tora*, alaternin and nor-rubrofusarin glucose, effectively scavenged $ONOO^-$ (Table 1). It would seem reasonable to use known $ONOO^-$, $\cdot NO$, and $\cdot O_2^-$ scavenging agents, penicillamine (Pannala et al 1997), carboxy-PTIO (Yoshida et al 1998) and Trolox (Satoh et al 1997), respectively, for comparative purposes in this study. Indirectly, the level of $ONOO^-$ can be controlled by levels of its precursors, $\cdot NO$ and $\cdot O_2^-$. Both alaternin and nor-rubrofusarin glucose not only directly scavenged $ONOO^-$ but also were involved in the inhibition of $\cdot O_2^-$ or $\cdot NO$ radical formation (Table 2), suggesting that the effects seem not to be simply due to $ONOO^-$ scavenging activity. They may be utilized in scavenging $ONOO^-$ in-vivo, where $ONOO^-$ is endogenously produced by the reaction between $\cdot O_2^-$ and $\cdot NO$. In this study, alaternin and nor-rubrofusarin glucose showed different capacity in scavenging $ONOO^-$. The phenomena might be explained in terms of chemical features (Figure 1). Namely, in contrast to natural flavonoids, which have several hydroxyl groups and display a wide range of antioxidative effects (Haenen et al 1997), alaternin has 4 potential hydroxyl groups while non-rubrofusarin only possesses two. This difference may account for their difference in $ONOO^-$ neutralization and consequently nitrotyrosine-formation reduction efficiency (Figure 2). Alaternin and nor-rubrofusarin glucose, major components in the seed extract of *Cassia tora*, have been reported to show a strong radical scavenging activity that affects pathological diseases and biological damage (Choi et al 1994, 2000).

Although a detailed mechanism by which each of these components scavenge $ONOO^-$ has yet to be clearly identified, two possible pathways may include a reaction between a phenolic $ONOO^-$ scavenger with $ONOO^-$ nitration or electron donation (Pannala et al 1998). Neither alaternin nor nor-rubrofusarin glucose reacted with $ONOO^-$ to produce a peak at 430 nm, implying that each compound underwent an electron donation to neutralize $ONOO^-$. At the same time, the decrease in absorbance at 430 nm strongly suggests that $ONOO^-$ scavengers inhibit the conversion of tyrosine into 3-nitrotyrosine induced by $ONOO^-$. In this study, both alaternin and nor-rubrofusarin glucose were examined for their involvement in the reaction of tyrosine with $ONOO^-$. Evidence indicated that the protective mechanism by which alaternin and nor-rubrofusarin glucose work was likely through reducing the availability of $ONOO^-$, rather than by blocking the formation of 3-nitrotyrosine by $ONOO^-$. This was confirmed by there being no increased absorbance of nitrotyrosine at 430 nm when either alaternin or nor-rubrofusarin glucose was exposed to $ONOO^-$ in the presence of tyrosine.

BSA, a protein abundant in plasma that is involved in the maintenance of colloid osmotic pressure and the transport of various ligands, was used as a model to probe $ONOO^-$protein reactivity. Western blot analysis of nitrated BSA indicated that the nitrotyrosine present in the protein sample was significantly inhibited by alaternin, but not by nor-rubrofusarin glucose. Protein tyrosine nitration by $ONOO^-$ may interfere with phosphorylation/dephosphorylation signalling pathways and alter enzyme functions (Li et al 1998; MacMillan-Crow & Thompson 1999; Viner et al 1999). Beckman & Koppenol (1996) demonstrated that nitrotyrosine forms in atherosclerotic lesions of human coronary arteries. In this regard, these findings, that pre-treatment with alaternin reduces the formation of nitrated BSA, are significant for therapeutical study.

In conclusion, we believe that both alaternin and nor-rubrofusarin glucose could prevent protein nitration and protect cells against the biological damage caused by $ONOO^-$ electron donation. The significant $ONOO^-$ scavenging property of alaternin could be useful in the prevention and treatment of $ONOO^-$-related diseases such as Alzheimer's disease, rheumatoid arthritis, cancer, inflammation and atherosclerosis. Further investigations, including in-vivo experiments, are suggested.

Conclusions

$ONOO^-$ is produced by the reaction of $\cdot O_2^-$ with ·NO. $ONOO^-$, a powerful oxidant, causes damage to proteins, lipids and DNA through nitration or oxidation. However, due to the lack of endogenous defence components against $ONOO^-$ in the body, the supplement of scavengers is needed.

In this study, evidence was obtained that alaternin in a natural herb is a potent scavenger of $ONOO^-$. Data were further confirmed by the finding that alaternin has a protective effect on bovine serum albumin (BSA) against $ONOO^-$.

Alaternin extracted from C*assia tora* showed the strongest $ONOO^-$ scavenging activity via electron donation mechanism. The significant $ONOO^-$ scavenging properties of alaternin could be useful for the prevention and treatment of $ONOO^-$-related diseases, such as Alzheimer's disease, rheumatoid arthritis, cancer, inflammation and atherosclerosis.

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