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Selective peroxynitrite scavenging activity of 3-methyl-1,2-cyclopentanedione from coffee extract

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Abstract

It has been known that reactive oxygen and nitrogen species such as nitric oxide (NO), superoxide radical ($^{\cdot}O_2^-$) and their byproduct peroxynitrite ($ONOO^-$) induce cellular and tissue injury, ultimately resulting in several human diseases. In this study, we examined scavenging effects of 3-methyl-1,2-cyclopentanedione (MCP) from coffee extract on the reactivity of those toxic molecules. MCP significantly inhibited both the oxidation of 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) by reactive oxygen species (ROS) (mainly $^{\cdot}O_2^-$) from kidney homogenate (41% at 100 μM) and the generation of fluorescent 4,5-diaminofluorescein (DAF-2) by NO from sodium nitroprusside (IC50 (concn producing 50% inhibition), 63.8 μM). More potently, however, MCP suppressed the oxidation of dihydrorhodamine 123 (DHR 123) to fluorescent rhodamine 123 mediated by authentic $ONOO^-$ with an IC50 value of 3.3 μM. The neutralizing effect of the reactivity of $ONOO^-$ by MCP was due to electron donation, not nitration of the compound. Additionally, MCP also decreased $ONOO^-$ formation of nitrotyrosine adducts of glutathione (GSH) reductase, and consequently protected the enzyme activity of GSH reductase against decreasing by $ONOO^-$, indicating that MCP may prevent $ONOO^-$-induced damage of GSH reductase. Furthermore, MCP only weakly suppressed NO production, which is one of the upstream sources of $ONOO^-$ in-vivo, suggesting that NO production may be not a pharmacological target for MCP. Taken together, our results suggest that MCP may be regarded as a selective regulator of $ONOO^-$-mediated diseases via direct scavenging activity of $ONOO^-$.

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Introduction

Reactive oxygen and nitrogen species (ROS and RNS) produced from various cells, including macrophages and neutrophils, have been reported to be effective materials against various foreign or infectious pathogens. Their overproduction, however, causes several diseases such as rheumatoid arthritis, transplanted organ rejection, atherosclerosis, amyotrophic lateral sclerosis, sepsis and aging (Squadrito & Pryor 1998). The reactive components include nitric oxide (NO), superoxide radical ($^{\cdot}O_2^-$) and their byproduct peroxynitrite ($ONOO^-$). Although the pathophysiological mechanisms by which the reactive species trigger cellular or tissue injury are not exactly elucidated, some reports have demonstrated the clear involvement of the reactive materials and their byproducts. For example, $ONOO^-$ is able to induce oxidation of thiol (-SH) groups on proteins, nitration of tyrosine, nitrosation (e.g. formation of *S*-nitrosoglutathione) and lipid peroxidation, causing cellular toxicity and affecting cell metabolism (Kooy et al 1994; Darley-Usmar & Halliwell 1996; Briviba et al 1999). Indeed, $ONOO^-$ induced nitrotyrosine has been identified in those pathological conditions (Nakazawa et al 2001). Furthermore, ROS and $ONOO^-$ are known to induce a variety of DNA modifications, including 8-nitroguanine formation and DNA strand breaks (Yermilov et al 1995, 1996).

Coffee has both pharmacologically hazardous and beneficial effects. Instant coffee exhibited direct genotoxic activity in several animal species (Duarte et al 1999). Treatment of supercoiled pBR 322 DNA with coffee caused single strain breaks due to ROS (Hiramoto 1998). By contrast, it was demonstrated that coffee extracts inhibit

platelet aggregation, suggesting an important therapeutic tool against generation of thrombosis in vascular disorders (Bydlowski et al 1987). Furthermore, coffee was shown to have in-vitro antioxidant activity, inhibiting lipid peroxidation and exerting a strong protective effect against mutagenicity and cytotoxicity (Stadler et al 1994). Although there is a growing understanding of the phenolic compounds from coffee on ROS scavenging activity, there is little information regarding the $ONOO^-$ neutralizing activity of other coffee ingredients.

The aim of this study is to define the effectiveness of 3-methyl-1,2-cyclopentanedione (MCP) from coffee extract as an antioxidant. To do this, a possible modulation of reactive species, including $ONOO^-$, and the major $ONOO^-$ production pathway by MCP was investigated using several in-vitro models and lipopolysaccharide (LPS)-activated RAW 264.7 cells. Our results suggest that MCP may be regarded as a selective regulator of $ONOO^-$-mediated diseases via direct scavenging activity.

Materials and Methods

Materials

3-Methyl-1,2-cyclopentanedione (MCP) (Figure 1; purity: more than 95%) was purified from coffee extract, as previously reported, and standard MCP was obtained from Aldrich (Milwaukee, WI). GSSG, NADPH, glutathione (GSH) reductase type II from wheat germ, nitro-L-arginine methyl ester (L-NAME), DL-penicillamine (DL-2- amino-3-mercapto-3-methylbutanoic acid) and lipopolysaccharide (LPS) were obtained from Sigma Chemical Co. (St Louis, MO). Dihydrorhodamine 123 (DHR 123) and 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) were from Molecular Probes (Eugene, OR) and $ONOO^-$ was from Cayman Chemical Co. (Ann Arbor, MI). 4,5-Diaminofluorescein (DAF-2) was purchased from Daiichi Pure Chemicals Co. (Tokyo, Japan). Polyvinylidene fluoride membrane (Immobilon-P) was obtained from Millipore Corp. (Bedford, MA) and chemiluminescence detection system was from Amersham Life Sciences, Inc. (Arlington Heights, IL). Antibody to nitrotyrosine was from Upstate Biotechnology (Lake Placid, NY). Enhanced chemiluminescence (ECL) Western blotting detection reagents were from Amersham Life Science (Buckinghamshire, UK). All other chemicals were of the highest purity available from Sigma Chemical Co. or Junsei Chemical Co. (Tokyo, Japan).

Measurement of $ONOO^-$ scavenging activity

$ONOO^-$ scavenging was measured by monitoring the oxidation of DHR 123 by modifying the method of Kooy et al (1994). A stock solution of 5 mM DHR 123 in dimethylformamide was purged with nitrogen and stored at $-20^{\circ}C$. Working solution with 5 μM DHR 123 diluted from the stock solution was placed on ice in the dark immediately before the study. Buffer (90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4) and 5 mM potassium chloride) was purged with nitrogen and placed on ice before use. Just

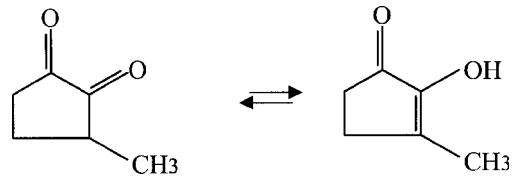

Figure 1 Structure of MCP from coffee extract.

before use, 100 μM diethylenetriaminepentaacetic acid (DTPA) was added. $ONOO^-$ scavenging by the oxidation of DHR 123 was measured on a microplate fluorescence spectrophotometer FL 500 (Bio-Tek Instruments, USA) 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 Ultraspec 2000 UV/visible spectrophotometer (Pharmacia-Biotech, UK).

Measurement of $^{\cdot}O_2^-$ scavenging activity

2,7-Dichlorodihydrofluorescein diacetate (H_2DCFDA) was oxidized to fluorescent 2,7-dichlorofluorescein (DCF) by ROS. The fluorescence intensity of DCF was measured by using a microplate fluorescence spectrophotometer FL 500 (Bio-Tek Instruments, USA) with excitation and emission wavelengths of 485 nm and 530 nm, respectively, for 1 h with or without the addition of kidney homogenate as an $^{\cdot}O_2^-$ source with kidney and 50 mM potassium phosphate buffer (pH 7.4).

Measurement of NO scavenging activity

NO scavenging was measured by monitoring DAF-2 by modifying the method of Chung et al 2001. 4,5-Diaminofluorescein (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). DAF-2 (1 mg) in 0.55 mL dimethyl sulfoxide was diluted with 50 mM phosphate buffer (pH 7.4) to 1/400 fold. A NO donor, 2 mM sodium nitroprusside, and 3.14 μM DAF-2 were 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 spectrophotometer (FL 500, Bio-Tek Instruments) at the excitation and the emission wavelengths of 485 nm and 530 nm after 10 min.

Interaction of MCP with $ONOO^-$

Spectrophotometric analysis was used to examine the mechanism of action of each sample with $ONOO^-$ (Pannala et al 1998). The spectral change in the visible region of

MCP in the presence of $ONOO^-$ was monitored at 430 nm to determine the existence of nitration. $ONOO^-$ (500 μM) in 0.3 M NaOH was added to a solution containing a sample in 50 mM phosphate buffer (pH 7.4), making a final volume of 1 mL. Each mixed solution was incubated at 37°C with shaking for 10 min and scanned between 190 nm and 600 nm on an Ultraspec 2000 UV-visible spectrophotometer (Pharmacia-Biotech, UK).

GSH reductase activity with $ONOO^-$ and MCP

Enzymatic activity of GSH reductase was determined by the NADPH reduction method (Mavis & Stellwagen 1968). MCP with 0.6 U mL^{-1} of GSH reductase was incubated with shaking at 37°C for 5 min. Then $ONOO^-$ was added, followed by another 10 min of incubation with shaking at 37°C. The test mixture was added to GSH reductase solution containing 1 mM GSSG, 0.09 mM β-NADPH, 0.13% (w/v) BSA in 75 mM potassium phosphate buffer and 2.6 mM EDTA (pH 7.4). GSH reductase activity was followed spectrophotometrically at 340 nm. Each assay was performed three times with similar results.

Sample preparation for the detection of nitrated protein

The sample was added to 25 μL of GSH reductase (25 mg protein/mL). The mixed samples were incubated with shaking at 37°C for 5 min. After $ONOO^-$ in 0.3 M NaOH was added, the samples were further incubated with shaking for 10 min at 37°C.

Western blot analysis

The prepared sample in gel loading buffer, pH 6.8 (12.5 mM Tris[hydroxymethyl]aminomethane, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 10% 2-mercaptoethanol and 0.2% bromophenol blue) in a ratio of 1:1 was boiled for 5 min. Total protein equivalents for each sample were separated on 8% SDS-polyacrylamide mini-gel using a Laemmli buffer system at 100 V and were transferred to a polyvinylidene difluoride (PVDF) membrane at 100 V for 1.5 h in a wet transfer system (BIO-RAD, Hercules, CA). The membrane was immediately placed in a blocking solution (5~10% non-fat dry milk in TBS-T buffer containing 10 mM Tris, 100 mM NaCl and 0.1% Tween 20, pH 7.5) at 4°C overnight. The membrane was washed in TBS-T buffer for 30 min and then incubated with a monoclonal anti-nitrotyrosine antibody (diluted 1:2000 in TBS-T buffer) at room temperature for 1~2 h. After three 15-min washings in TBS-T buffer, the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody at room temperature for 1~2 h. After four 15-min washings in TBS-T buffer, antibody labelling was detected using ECL per manufacturer's instruction and exposed to radiographic film. Pre-stained blue protein markers were used for molecular-weight determination.

Cell culture

RAW 264.7 cells were obtained from American Type Culture Collection (Rockville, MD). The cells were cultured in DMEM medium (Nissui Co., Tokyo, Japan) supplemented with 10% heat-inactivated (56°C for 30 min) fetal serum (Gibco, Grand Island, NY), 233.6 mg mL^{-1} glutamine, 0.25 μg mL^{-1} amphotericin B and 72 μg mL^{-1} gentamicin (Sigma), and adjusted to pH 7.4–7.6 with $NaHCO_3$ in an atmosphere of 5% CO_2. The fresh medium was replaced after one day to remove nonadherent cells or cell debris.

Quantification of nitrite and nitrate

RAW 264.7 cells in a 24-well plate were pre-incubated for 24 h. After pre-incubation with or without MCP for 3 h, cells were treated with 100 ng mL^{-1} LPS for 24 h. The collected media was stored at −20°C for measurements of the NO metabolites NO_2^- and NO_3^- (NOx). Before measuring NOx levels, samples were deproteinized by adding an equal volume of methanol, and then centrifuged (5000 *g*, 10 min). The assay was carried out by NO-analysing system (ENO-20, Eicom Corp., Kyoto, Japan) (Kimura et al 1999). In brief, nitrite and nitrate were separated on a polystyrene polymer column, and the nitrate was reduced by passage through a cadmium column. Resulting nitrite was mixed with a Griess reagent (Green et al 1982) to form a purple azo dye. The absorbance of the product dye at 540 nm was measured by a flow-through spectrophotometer.

Statistical analysis

Data are expressed as mean±standard deviation (s.d.). Results were analysed statistically by Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Antioxidative activity of MCP

Coffee has been reported to display strong scavenging activity on the reactivity of $ONOO^-$. MCP is one of the

Table 1 The molar concentrations (μM) of MCP producing 50% inhibition (IC50) on scavenging activity.

	$\cdot O_2^-$	NO	$ONOO^-$
MCP	≫100	63.80±1.45	3.26±0.50
Trolox[a]	27.29±0.69		
Carboxy-PTIO[a]		0.32±0.02	
Penicillamine[a]			3.03±0.38

Data are mean±s.d. of triplicate measurements. [a]Used as a positive control.

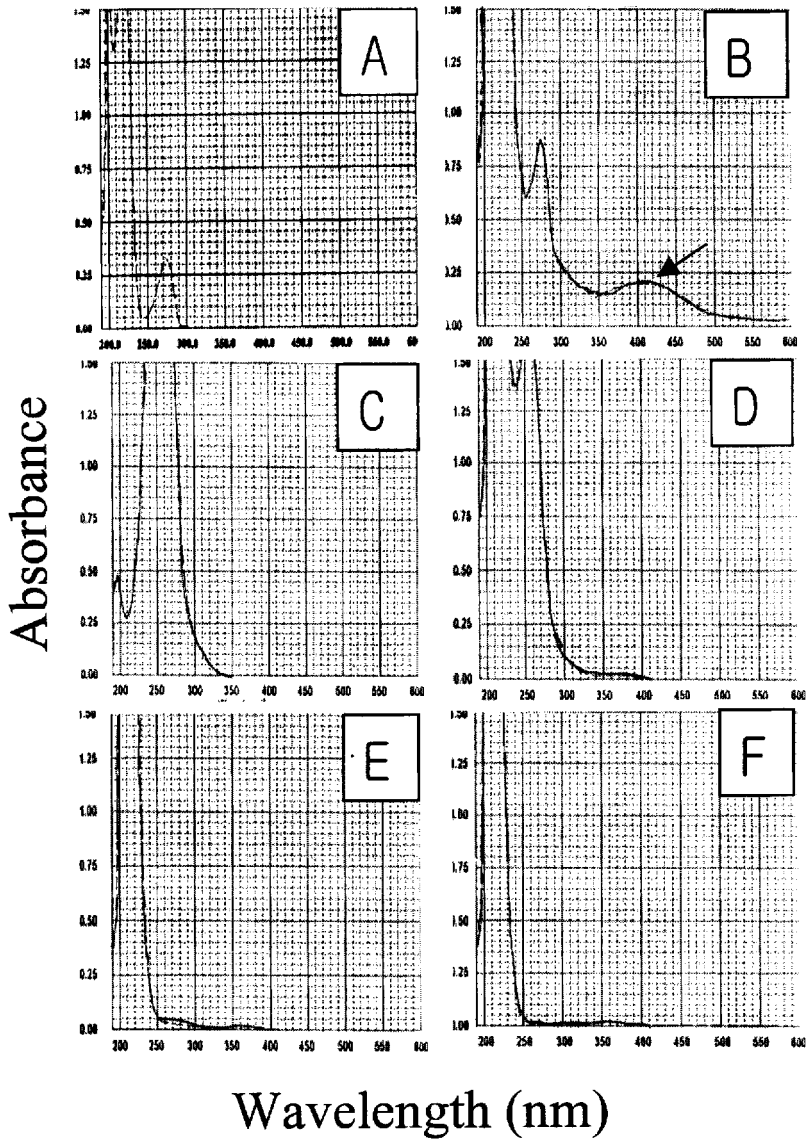

Figure 2 MCP neutralizes the reactivity of $ONOO^-$ via electron donation. Tyrosine (500 μM) was incubated without (A) or with $ONOO^-$ (500 μM) (B) at 37°C for 10 min. MCP (500 μM) alone (C) or different doses (500 (D), 100 (E), and 20 (F) μM) of MCP were incubated with $ONOO^-$ (500 μM) at 37°C for 10 min. The spectrophotometric analysis was performed as described in Material and Methods. The arrow indicates a nitration peak at around 400–450 nm.

components in coffee extract, but has not yet been evaluated for antioxidative properties, including other biological activity. Therefore, in this study, we carefully examined whether MCP acts as an active principle from coffee extract. To do this, we first tested radical scavenging activity on the reactivity of ROS and RNS. MCP significantly inhibited the oxidation of H_2DCFDA by ROS from kidney homogenate and the generation of fluorescent DAF-2 by NO from sodium nitroprusside (Table 1). The inhibitory activity of MCP at 100 μM was 41% (ROS) and 75% (NO), which was relatively weaker than that of positive controls (trolox IC50 (concn producing 50% inhibition), 27.3 μM; carboxy-PTIO IC50, 0.3 μM). Unexpectedly, MCP potently suppressed the oxidation of DHR 123 to fluorescent rhodamine 123 mediated by authentic $ONOO^-$ with an IC50 value of 3.3 μM, similar to penicillamine, a well-known $ONOO^-$ scavenger (IC50, 3.0 μM).

Interaction of MCP with $ONOO^-$

To explore the scavenging mechanism of MCP by which it undergoes either nitration reaction or electron donation after the addition of $ONOO^-$, a spectrophotometric analysis at 400–450 nm was performed. Figure 2(A, B) shows that tyrosine undergoes nitration by $ONOO^-$, because there is a peak around 430 nm. MCP alone did not produce the peak around 430 nm (Figure 2C) and in contrast to tyro-

sine, after interaction with $ONOO^-$, MCP did not display any spectrometric change around 430 nm with different concentration (Figure 2D–F), strongly indicating that electron donation, but not nitration, may be regarded as the neutralizing mechanism.

Effects of MCP on inactivation and nitration of GSH reductase by $ONOO^-$

$ONOO^-$ modifies the activity of several enzymes in which thiol groups are essential for catalytic function (Di Simplicio et al 1998). Indeed, the activity of GSH reductase, a rate limiting enzyme, was markedly decreased after interaction with $ONOO^-$ in a dose-dependent manner (Figure 3A) and, simultaneously, the nitrotyrosine level determined by anti-nitrotyrosine antibody was also increased (Figure 3C) as reported previously (Chung et al 2001). We next addressed whether MCP is able to modulate inactivation of GSH reductase and its nitration induced by $ONOO^-$ under the same conditions. As expected, MCP attenuated $ONOO^-$-induced inactivation of GSH reductase in a dose-dependent manner (Figure 3B). Thus, $ONOO^-$ suppressed GSH reductase activity (from 20.3 mU to12.3 mU), whereas MCP (100 μM) protected the enzyme from $ONOO^-$-induced inactivation (from 12.3 mU to 17.3 mU). MCP also blocked the formation of nitrotyrosine in GSH reductase triggered by $ONOO^-$ (Figure 3D). These results indicate that MCP may protect significantly the oxidative damage of GSH reductase.

The nitrate and nitrite levels in medium

Although MCP showed weak inhibitory activity on SNP-generated NO, it might be important to test whether MCP is capable of regulating NO production (in activated macrophages), by which $ONOO^-$ is generated in-vivo. NO inhibitory activity was determined by analysing nitrite (NO_2) and nitrate (NO_3) levels. Figure 4 shows that MCP only slightly inhibited the production of NOx (NO_2/NO_3)

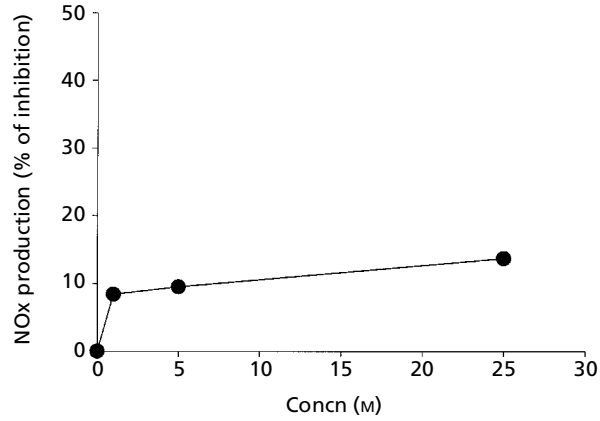

Figure 4 Effect of MCP on NO_2^- and NO_3^- (NOx) production in LPS-induced RAW 264.7 cells. The cells were pretreated with different concentrations of MCP for 3 h and incubated with LPS (100 ng mL^{-1}) for 24 h. The levels of NOx were determined by Griess reaction as described in Materials and Methods. Each assay was performed by three separate experiments with similar results.

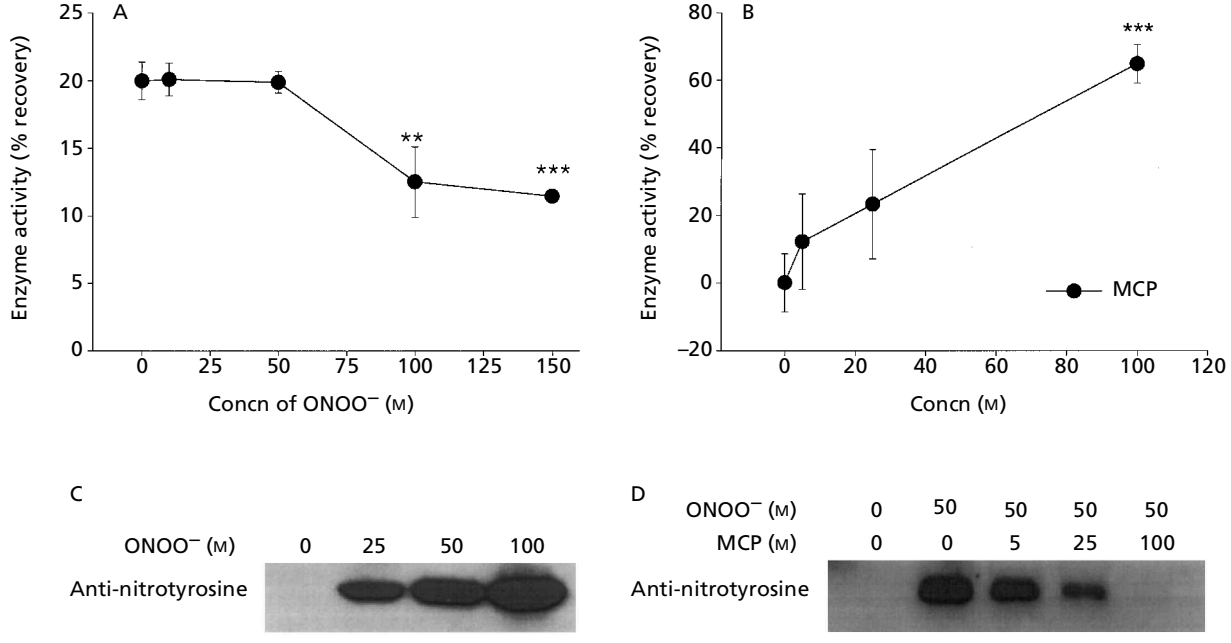

Figure 3 MCP abolishes inactivation of GSH reductase by $ONOO^-$ via suppression of nitrotyrosine formation. The enzymatic change of GSH reductase activity induced by $ONOO^-$ in the absence (A) or presence (B) of MCP was determined by NADPH reduction method as described in Materials and Methods. The nitrotyrosine level of GSH reductase triggered by $ONOO^-$ in the absence (C) or presence (D) of MCP was analysed by Western blot with antibody specific for nitrotyrosine. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with normal or control.

in RAW 264.7 cells after LPS treatment (13.7% at 50 μM), whereas positive control drug, L-NAME, dose-dependently decreased NO production with an IC50 value of 195.4 μM. At all tested concentrations, MCP was not cytotoxic (data not shown). Therefore, this result suggests that cellular NO production from macrophages is unable to be modulated by MCP treatment.

Discussion

In contrast to $^{\cdot}O_2^-$ and NO, their byproduct $ONOO^-$ is a relatively stable and non-radical oxidant, involved in several disease states such as chronic or acute inflammation (Stamler et al 1992; Huie & Padmaja 1993). The ability of $ONOO^-$ to oxidize or nitrate tyrosine residues on a variety of proteins contributes to $ONOO^-$-mediated cellular dysfunction (Stamler 1994; Kooy et al 1997; Mondoro et al 1997). Due to the lack of endogenous enzymes responsible for $ONOO^-$ inactivation, developing specific $ONOO^-$ scavengers is an important strategy.

Our study shows that MCP from coffee not only strongly scavenges $ONOO^-$, but also effectively protects a cellular enzyme (GSH reductase) from the detrimental effects of $ONOO^-$, rather than acting as a single radical scavenger for ROS and RNS. Thus, the scavenging effect of MCP on the reactivity of $^{\cdot}O_2^-$ and NO was relatively very weak, compared with the effects on the reactivity of $ONOO^-$ (the IC50 values are summarized in Table 1), suggesting that the effects seem not to be simply due to radical scavenging activity. The phenomena might be explained in terms of chemical features (Figure 1). Namely, in contrast to naturally occurring flavonoids (such as quercetin) or other compounds (such as 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) and hydroquinone) having several hydroxyl groups and displaying a wide range of antioxidative effects (Haenen et al 1997; Chung et al 2001), MCP only possesses one potential hydroxyl group induced by chemical conversion between two carbonyl groups which may be responsible for neutralizing the reactivity of $ONOO^-$. Furthermore, the structural characteristics of MCP seem to be beneficial in that some flavonoids which have an *ortho*-trihydroxyl group (such as delphinidine, myricetic and quercetagetin) act as potential oxidants that are able to induce DNA breakage (Oshima et al 1998).

The mode of $ONOO^-$ scavenging action by antioxidants has not been fully defined. In the case of phenolic compounds having hydroxyl groups, however, either nitration or electron donation during reaction with $ONOO^-$ has been proposed (Pannala et al 1997, 1998), and those phenomena could be distinguished by spectrophotometry at around 400–450 nm. Thus, the conversion of a compound into a nitro-form by $ONOO^-$ gives it a peak at 430 nm, driven from the breakdown of $ONOO^-$ to nitrogen dioxide radical ($^{\cdot}NO_2$) or the nitronium ion (NO_2^+). While tyrosine and hydroquinone (submitted for publication) were shown to be nitrated by $ONOO^-$, Figure 2 apparently shows that the neutralizing mechanism by which MCP suppresses the $ONOO^-$ reactivity was due to electron donation, not nitration. Although the intermediate form of MCP after electron donation is not fully identified, it seems that the intermediate form was not toxic, because MCP treatment up to 100 μM did not change cell viability of RAW 264.7 cells following LPS stimulation under which ROS and RNS are produced. In terms of potential adverse effect, therefore, it is regarded that MCP may be a potent, but safe drug, unlike some antioxidants that display strong oxidative and cytotoxic effects (Ohshima et al 1998; Shi et al 2001) as mentioned above. Further experiments to demonstrate the toxicity of MCP should be followed.

$ONOO^-$ could induce a functional damage of some biological molecules such as bovine serum albumin and low-density lipoprotein via nitrotyrosine (Chung et al 2001). Nitration of protein has been reported in various hypertensive disorders, neurologic disorders and chronic renal disease as the footprint of $ONOO^-$ (Vaziri et al 1999, 2002; Roberts et al 2000; Deng et al 2001). We, and other groups, also found that GSH reductase, a biological defence enzyme against some free radicals (Rahman et al 1999), involved in maintaining intracellular balance of GSH, undergoes $ONOO^-$-induced inactivation via formation of nitrotyrosine (Figure 3A, C) (Francescutti et al 1996; Sies et al 1998). The scavenging property of MCP seems to be linked to protecting $ONOO^-$-induced damage of cellular defence proteins, such as GSH reductase, because MCP suppressed the increase of nitrotyrosine levels following $ONOO^-$ treatment and protected enzyme from the decreased enzymatic activity in a dose-dependent manner (Figure 3). Thus, this result strongly suggests that MCP may regulate cellular defence mechanisms against oxidative stress induced by some radicals through maintaining GSH reductase activity, leading to retaining proper GSH levels. Furthermore, considering that protein tyrosine nitration by $ONOO^-$ interferes with phosphorylation/dephosphorylation of some signalling molecules as well as altering enzyme functions (Li et al 1998; MacMillan-Crow & Thompson 1999), MCP may be supposed to display protective effects toward these phenomena. Indeed, we have demonstrated that $ONOO^-$ triggered cell apoptosis (Sandoval et al 1997) via blocking cellular biochemical pathways and a potent $ONOO^-$ scavenger, TDB, showed a strong cytoprotective effect. Thus, it would be of interest to see whether MCP is capable of modulating other $ONOO^-$-mediated toxicological effects (Chung et al 2001). Taken together, it is worth considering that MCP may directly neutralize $ONOO^-$-mediated toxicity through electron donation and further maintain the level of cellular defence enzymes, such as GSH reductase, via blocking $ONOO^-$-induced damage.

The main in-vivo source of $ONOO^-$ is known to be the NO synthesis pathway mediated by inducible nitric oxide synthase (Oldreive & Rice-Evans 2001). To examine the possibility that MCP is able to modulate cellular $ONOO^-$ formation, we tested the inhibitory effect of MCP on NO production from LPS-activated macrophage RAW 264.7 cells. Figure 4 shows that MCP only slightly suppressed NOx production, even at high concentrations, suggesting that MCP is not effective in the upstream pathway for $ONOO^-$ production, although it is unclear whether or not MCP is unable to penetrate into membrane or there are

no MCP target molecules in the NO-producing pathway. Furthermore, the weak inhibitory effect of MCP seems to be due to direct NO scavenging activity, according to DAF assay.

In conclusion, MCP from coffee extract may be regarded as a potent regulator of $ONOO^-$-mediated diseases via direct scavenging activity of the reactivity of $ONOO^-$. The scavenging activity may be mainly mediated by electron donation. Due to the lack of endogenous enzymes responsible for $ONOO^-$ inactivation, developing specific $ONOO^-$ scavengers is considerably important. Therefore, our data suggest that MCP is an active coffee extract that is a new, potent and selective scavenger of $ONOO^-$.

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