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Evaluation of Antioxidants Using an Arachidonic Acid-Stimulated Platelet Electron Spin Resonance System

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ABSTRACT

A novel cell-containing assay system using electron spin resonance (ESR) and spin-trapping methods was established. We used this assay system to evaluate the antioxidant activities of various dietary antioxidants including resveratrol, lycopene, quercetin and rutin. Resveratrol showed the strongest radical-scavenging activity among the compounds tested. Our results provided useful data for the establishment of a model for evaluating the antioxidant activities of natural dietary substances in cell-containing systems.

Key words: arachidonic acid, platelets, electron spin resonance, hydroxyl radical, carbon-centered radical

INTRODUCTION

Reactive oxygen species (ROS) are generated in living cells via both enzymatic and non-enzymatic mechanisms. Some ROS are required for the presentation of specific physiological functions, and ROS generation was implicated in the pathogenesis of a number of diseases[(1)]. ROS are also generated by arachidonic acid (AA) metabolites, which are released from cell membranes. The AA-induced apoptotic death of various tumor cells is characteristic of ROS generation[(2-5)]. AA-induced ROS generation may be via oxidative metabolic processes by cyclooxygenase (COX) and lipoxygenase (LOX)[(6)]. AA was also reported to induce ROS formation through NADPH oxidase[(7,8)].

Antioxidants may react with ROS and terminate their injurious actions, and for that reason, many scientists are searching for novel dietary antioxidants. Electron spin resonance (ESR) is a spectroscopic technique that directly detects free radicals and is widely applied to evaluate free radical-scavenging activities of antioxidants in cell-free systems using a stable free radical or radical-generating system[(9)]. However those assay systems are not conducted under physiological conditions and the results may not reveal the actual actions of the tested substances *in vivo*[(9)]. The EPR technique is also applied in many studies with various cell lines to estimate the free radical scavenging capacities of antioxidants[(10,11)] and to better understand the role of free radicals in many biological mechanisms[(12,13)]. We attempted to establish a cell-containing assay system using ESR and spin-trapping methods.

Human platelet suspensions are a widely used experimental tool because they are easy to prepare. ROS were suggested to be "second messengers" during platelet activation[(14)]. A previous study showed that collagen-induced platelet aggregation is associated with superoxide anion and hydroxyl radical generation, which is dependent on endogenous AA release and metabolism[(15)]. Leo *et al.* demonstrated that platelets exposed to anoxia-reoxygenation intrinsically generated superoxide anions and hydroxyl radicals, which in turn activate endogenous AA metabolism via phospholipases A2 and C[(16)]. Singh *et al.* demonstrated that the addition of exogenous AA generated hydroxyl radicals via the LOX pathway in human platelets[(17)], whereas Zuo *et al.* showed that superoxide anion release through LOX activity is the major source of extracellular ROS in skeletal muscles[(18)]. We propose that superoxide anions may be the primary species produced by the addition of exogenous AA to human platelets and are converted to hydrogen peroxide by superoxide dismutase (SOD) catalysis and switched to hydroxyl radicals via Fenton's reaction.

In this study, we investigated the mechanisms of

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AA-induced ROS generation in human platelets. Our results suggested that AA generated ROS primarily via 12-LOX and generated a carbon-centered radical via COX in human platelet suspensions. These results may provide useful data to establish a model for evaluating the antioxidant activities of natural dietary substances in cell-containing systems.

MATERALS AND METHODS

I. *Materials*

AA, bovine serum albumin (BSA), clotrimazole, 5,5-dimethyl-1 pyrroline N-oxide (DMPO), diphenyliodonium, indomethacin, lycopene, nordihydroguaiaretic acid (NDGA), quercetin, resveratrol, rutin, sodium azide (NaN_3) and sodium citrate were purchased from Sigma Chemical (St. Louis, MO, USA). Diethyldithiocarbamate (DETC) was purchased from Aldrich (Milwaukee, WI, USA). 5-Diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) was purchased from Alexis Biochemical (Lausen, Switzerland). Hinokitiol was purchased from Wako Pure Chemical Industries (Osaka, Japan). The COX-1 monoclonal antibody (anti-mouse) and 12-LOX (murine leukocyte) polyclonal antiserum (anti-rabbit) were purchased from Abcam (Cambridge, MA, USA).

II. *Preparation of Human Platelet Suspensions*

Human platelet suspensions were prepared as previously described[19]. In this study, the human volunteers gave informed consent. In brief, blood was collected from healthy human volunteers who had not taken medicine during the preceding 2 weeks, and was mixed with acid/citrate/glucose. After centrifugation at 120 ×g for 10 min at room temperature, the supernatant (platelet-rich plasma; PRP) was supplemented with prostaglandin (PGE_1 (0.5 μM) and heparin (6.4 IU/mL), then incubated for 10 min at 30°C and centrifuged at 500 ×g for 10 min. Washed platelets were finally suspended in Tyrode's solution containing BSA (3.5 mg/mL) and adjusted to a concentration of 4.5×10^8 platelets/mL. The final concentration of Ca^{2+} in Tyrode's solution was 1 mM.

III. *Measurement of Free Radicals in Platelet Suspensions by ESR Spectrometry*

The ESR method used a Bruker EMX ESR spectrometer as described previously[20], but with some modifications. ESR spectra were recorded at room temperature using a quartz flat cell designed for aqueous solutions. The dead time of sample preparation and ESR analysis was exactly 30 s after the last addition. Conditions of ESR spectrometry were as follows: 20 mW power at 9.78 GHz, with a scan range of 100 G, and a receiver gain of 6.32×10^4. The modulation amplitude, sweep time and time constant are given in the legends to the figures and tables.

(I) *Free Radicals Induced by AA in the Presence of DMPO*

Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of AA (10 μM).

(II) *Free Radicals Induced by AA in the Presence of DEPMPO*

Human platelets (1.5×10^8 platelets/mL) preincubated with DEPMPO (50 mM) followed by the addition of AA (10 μM).

(III) *Effects of Various Enzyme Inhibitors on AA-induced Free Radicals*

Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of AA (10 μM). Emzyme inhibitors were added 10 min before the addition of AA.

(IV) *Effects of 12-LOX Inhibition on AA-induced Free Radicals*

Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of AA (10 μM) and hinokitiol (20 μM), a 12-LOX inhibitor, or addition of AA (10 μM) and 12-LOX antibody (1.5×10^{-3} mg/mL).

(V) *Effects of Dietary Antioxidants on AA-induced Free Radicals*

Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of AA (10 μM) and resveratrol (1 μM), lycopene (6 μM), rutin (150 μM), and quercetin (25 μM).

(VI) *Effects of Dietary Antioxidants on AA-induced g = 2.005 Radical*

Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of AA (10 μM) and 12-LOX antibody (1.5×10^{-3} mg/mL). Resveratrol (5, 12 μM), lycopene (12 μM), rutin (150 μM) and quercetin (25 μM) were added 10 min before the addition of AA.

IV. *Statistical Analysis*

Experimental results are expressed as the mean ± SEM and are accompanied by the number (*n*) of observations. Data were assessed using analysis of variance (ANOVA). If this analysis indicated significant differences among the group means, then each group was compared using the Newman-Keuls method. A p value of less than 0.05 was considered statistically significant.

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RESULTS

I. *ESR Investigations of Free Radicals Induced by AA in the Presence of DMPO*

We demonstrated that AA produced a typical 4-line hydroxyl radical signal (aN = aH = 14.8 G) detectable by spin trapper DMPO (200 mM) in human platelet suspensions (Figure 1B). The dose-response study showed that the DMPO-OH signals were detected at rest because resting platelets contain a small amount of free AA[21]. When 10 nM of AA was added, the signal obviously increased, and a peak was reached with 10 μM of AA, after which it began to gradually decrease (Figure 3). Incubation with the SOD inhibitor,

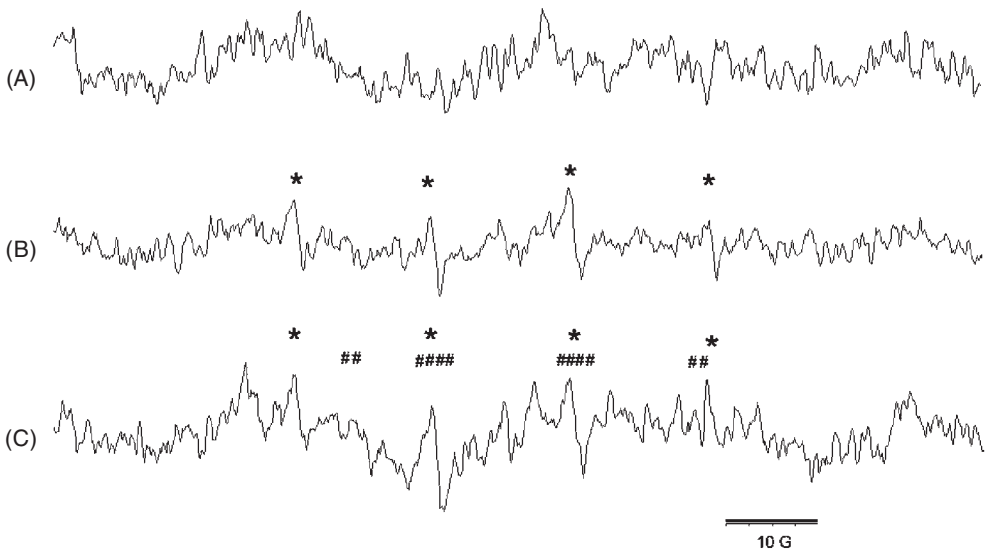

Figure 1. ESR spectra obtained from the reaction of a human platelet suspension in the presence of DMPO. Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM) followed by the addition of (A) a blank, (B) arachidonic acid (AA) (10 μM), or (C) AA (10 μM) and DETC (100 μM). Instrument parameters were as follows: a modulation amplitude of 1 G, a time constant of 164 ms and scanning for 42 s with 8 scans accumulated. The ESR spectra are labeled to show their components: DMPO-OH adduct (∗) and DMPO-OOH adduct (#).

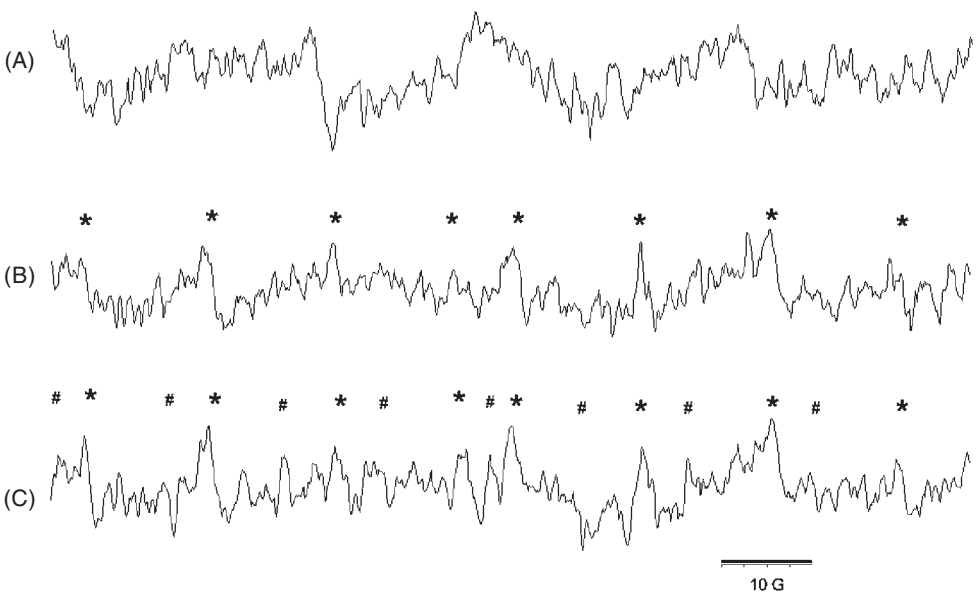

Figure 2. ESR spectra obtained from the reaction of a human platelet suspension in the presence of DEPMPO. Human platelets (1.5×10^8 platelets/mL) were preincubated with DEPMPO (50 mM) followed by the addition of (A) a blank, (B) arachidonic acid (AA) (10 μM), or (C) AA (10 μM) and DETC (100 μM). The instrument parameters were exactly the same as those given in Figure 1. The ESR spectra are labeled to show their components: DEPMPO-OH adduct (∗) and DEPMPO-OOH adduct (#).

DETC (100 μM), resulted in an ESR spectrum containing contributions from both superoxide radical adducts and hydroxyl radical adducts (Figure 1C). However the superoxide radical signals were not so obvious. This may be due to the short half-life of the DMPO-OOH spin adduct, which spontaneously decays to form the DMPO-OH adduct[22]. The assignment of these adducts were made on known hyperfine couplings[23].

II. *ESR Investigations of Free Radicals Induced by AA in the Presence of DEPMPO*

AA (10 μM) produced an 8-line hydroxyl radical signal detectable by spin trapper DEPMPO (50 mM) in human platelet suspensions (Figure 2B). The addition of DETC (100 μM) resulted in an ESR spectrum containing contributions from two spin adducts which were assigned as superoxide radical adducts and hydroxyl radical adducts (Figure 2C) according to the known hyperfine couplings[24]. It was reported that the DEPMPO-OOH spin adduct is significantly more persistent (15 times at pH 7) than the DMPO-OOH spin adduct[23], and it does not unexpectedly decay to form the DEPMPO-OH adduct[25]. Therefore, the more-apparent spectra of the DEPMPO-OOH adducts were observed under the same experimental conditions (Figure 2C).

III. *Mechanism Studies on the Generation of Hydroxyl Radicals by AA-stimulated Human Platelets*

Formation of the hydroxyl radical signal was monitored following the addition of several inhibitors to 150 μL of platelet suspensions. The inhibitors were added 10 min before the addition of AA. Table 1 shows the effects of various inhibitors on AA (10 μM)-stimulated human platelets. The hydroxyl radical signal formed by 10 μM of AA was arbitrarily designated 100% and was respectively inhibited to 44.3% and 58.8% by 10 μM of hinokitiol, a selective inhibitor of the platelet-type isozyme of arachidonate 12-LOX[26], and 30 μM of NDGA, a nonselective inhibitor of LOX[27]. These results indicate that the AA-induced hydroxyl radical generation is mainly associated with 12-LOX in human platelets. In contrast, 10 μM of indomethacin, a COX inhibitor, and 1 mM of NaN_3, a heme protein inhibitor, significantly enhanced the formation of the hydroxyl radical signal to 126.8% and 143.9%, respectively. These results are possibly due to the inhibition of COX and peroxidase activities, thus enhancing the accumulation of AA, leading to increased formation of hydroxyl radicals via 12-LOX. Furthermore, 100 μM of diphenyliodonium, an NADPH oxidase inhibitor, showed no significant effect on radical formation.

IV. *Generation a g = 2.005 Signal in AA-stimulated Human Platelets by Inhibition of 12-LOX*

The AA-induced hydroxyl radical signal by spin trapper DMPO in human platelet suspensions was inhibited by a 12-LOX inhibitor and by a 12-LOX antibody, which results in the sequential generation of the $g = 2.005$ doublet signal (Figure 4C, D). The $g = 2.005$ doublet signal obviously increased when the concentration of AA increased (Figure 4E). The addition of ascorbic acid abolished the $g = 2.005$ radical

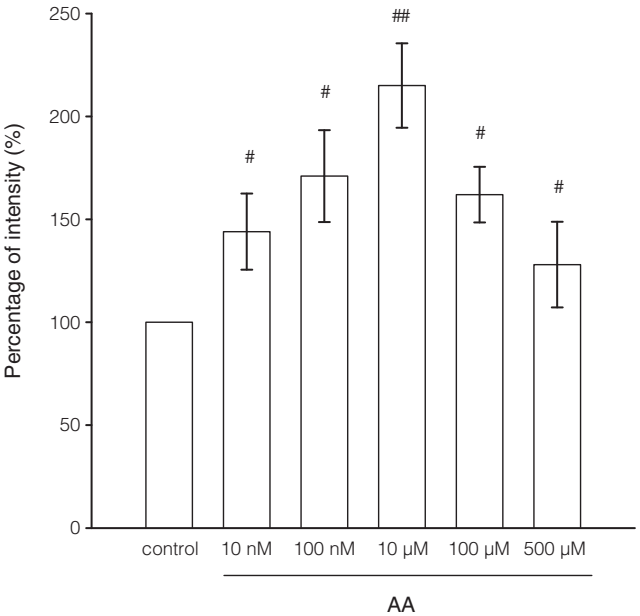

Figure 3. The percentage intensities of different concentrations of arachidonic acid (AA)-induced hydroxyl radicals in washed human platelets. Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM), followed by the addition of alcohol (control), AA (10 nM), AA (100 nM), AA (10 μM), AA (100 μM), or AA (500 μM) to trigger platelet activation. The instrument parameters were exactly the same as those given in Figure 1. ($^{\#}p < 0.05$; $^{\#\#}p < 0.005$, n = 3).

Table 1. Effects of various enzyme inhibitors on the intensity of 10 μM arachidonic acid (AA)-induced hydroxyl radicals in washed human platelets

Sample	Percent of control Value (%) ± SEM (n = 4)
PS	74.9 ± 6.7
PS + 10 μM of AA (control)	100.0 ± 0.0
Control + 10 μM of indomethacin	126.8 ± 8.8*
Control + 1 mM of NaN_3	143.9 ± 16.8*
Control + 10 μM of hinokitiol	44.3 ± 5.8*
Control + 30 μM of NDGA	58.8 ± 4.5*
Control + 100 μM of diphenyliodonium	117.2 ± 7.0

The reaction conditions and techniques of the ESR measurements are described in "Materials and Methods". Enzyme inhibitors were added to the platelet suspension (PS) (1.5×10^8 platelets/mL) preincubated with DMPO (200 mM) in the presence of 10 μM of AA. The instrumental parameters were exactly the same as those given in Figure 1. All values were normalized to 100% for 10 μM of AA and represent the average of 2 independent incubations. Data are presented as the mean ± SEM. (* $p < 0.05$).

to lead to the sequential generation of the small doublet (aH = 1.82 G) of the ascorbyl free radical (Figure 4F), indicating that the $g = 2.005$ radical was not the ascorbyl radical.

V. *Effects of Dietary Antioxidants on the Intensity of the Hydroxyl Radical Induced by 10 μM of AA in Human Platelets*

Formation of the AA-induced hydroxyl radical was monitored following the addition of several natural dietary antioxidants to 150 μL of platelet suspensions. The antioxidants were added 10 min before the addition of AA. Table 2 shows the effects of various antioxidants in AA (10 μM)-stimulated human platelets. The hydroxyl radical signal formed by 10 μM of AA was arbitrarily designated 100% and was significantly inhibited by resveratrol (1 μM), lycopene (6 μM), rutin (150 μM) and quercetin (25 μM). Resveratrol showed the strongest hydroxyl radical-scavenging activity among the tested compounds.

VI. *Effects of Dietary Antioxidants on the Intensity of the g = 2.005 Radical Induced by the Addition of a 12-LOX Antibody to AA-stimulated Human Platelets*

Formation of the $g = 2.005$ signal radical was monitored, following the addition of several natural antioxidants to 150 μL of platelet suspensions. The antioxidants were added 10 min before the addition of AA. Table 3 shows the effect of

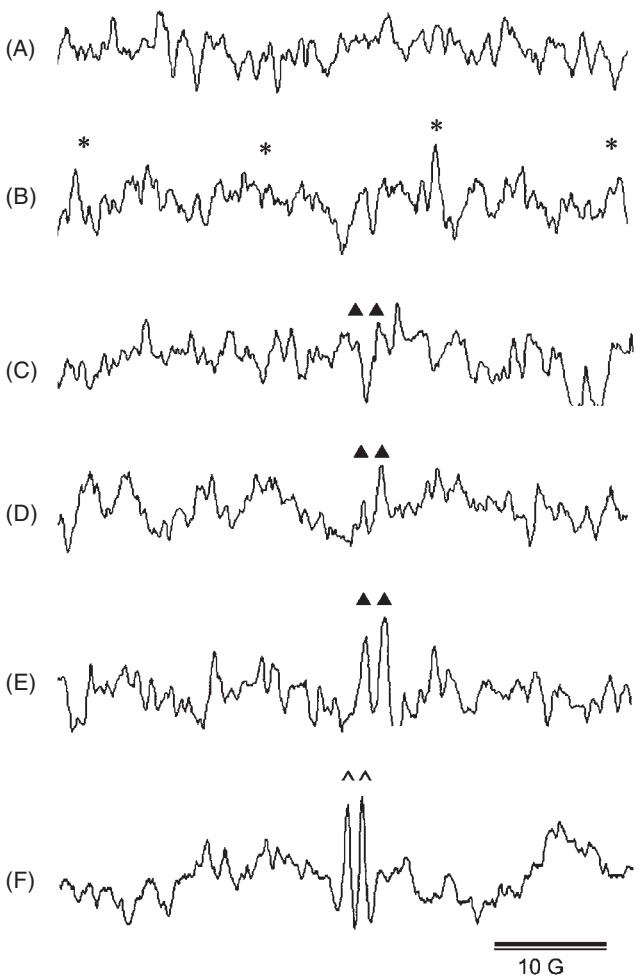

Figure 4. ESR spectra obtained from the reaction of a human platelet suspension in the presence of DMPO. Human platelets (1.5×10^8 platelets/mL) were preincubated with DMPO (200 mM) followed by the addition of (A) a blank, (B) arachidonic acid (AA) (10 μM) (C) AA (10 μM) and hinokitiol (20 μM), (D) AA (10 μM) and a 12-LOX antibody (1.5×10^{-3} mg/mL), (E) a 12-LOX antibody (1.5×10^{-3} mg/mL) and AA (100 μM), or (F) ascorbic acid (300 μM) and a 12-LOX antibody (1.5×10^{-3} mg/mL) and 10 μM of AA. The ESR spectra are labeled to show their components: DMPO-OH adduct (∗), $g = 2.005$ radical (▲), and ascorbyl radical (^).

Table 2. Effects of antioxidants on the intensity of hydroxyl radicals induced by 10 μM of arachidonic acid (AA) in washed human platelets

Sample	Percent of control Value (%) ± SEM (n = 4)
PS + 10 μM of AA (control)	100.0 ± 0.0
Control + 1 μM of resveratrol	56.5 ± 3.7**
Control + 6 μM of lycopene	57.0 ± 9.0*
Control + 150 μM of rutin	57.3 ± 8.0*
Control + 25 μM of quercetin	59.9 ± 9.2*

The reaction conditions and techniques of ESR measurements are described in "Materials and Methods". Antioxidants were added to the platelet suspension (PS) (1.5×10^8 platelets/mL) preincubated with DMPO (200 mM) in the absence or presence of 10 μM of AA. The instrumental parameters were exactly the same as those given in Figure 1. All values were normalized to 100% for the control and represent the average of four independent incubations. Data are presented as the mean ± SEM. (* $p < 0.05$; ** $p < 0.005$).

Table 3. Effects of antioxidants on the intensity of the g = 2.005 radical induced by a 12-LOX antibody in arachidonic acid (AA)-stimulated human platelets

Sample	Percent of control Value (%) ± SEM (n = 4)
P S (blank)	49.1 ± 19.2
PS + 10 μM of AA	48.4 ± 9.2
PS + 10 μM of AA + 12-LOX-antibody (control)	100.0 ± 0.0
Control + 5 μM of resveratrol	62.1 ± 4.9*
Control + 12 μM of resveratrol	52.5 ± 15.8*
Control + 12 μM of lycopene	57.6 ± 9.5*
Control + 150 μM of rutin	57.6 ± 11.7*
Control + 25 μM of quercetin	53.1 ± 20.8*

The reaction conditions and techniques of ESR measurements are described in "Materials and Methods". Antioxidants were added to the platelet suspension (PS) (1.5×10^8 platelets/mL) preincubated with DMPO (200 mM) in the absence or presence of 10 μM of AA and a 12-LOX antibody (1.5×10^{-3} mg/mL). The instrumental parameters were exactly the same as those given in Figure 1. All values were normalized to 100% for the control and represent the average of four independent incubations. Data are presented as the mean ± SEM. (* $p < 0.05$).

various dietary antioxidants in AA and 12-LOX antibody-treated human platelets. The $g = 2.005$ radical signal formed by 10 μM of AA and the 12-LOX antibody (1 mg/mL, 1.5 μL) was arbitrarily designated 100% and was significantly inhib ited by resveratrol (5, 12 μM), lycopene (12 μM), rutin (150 μM) and quercetin (25 μM). Resveratrol showed the strongest $g = 2.005$ signal radical-scavenging activity among the tested compounds.

DISCUSSION

Diet-derived antioxidants may play an important role in protecting against chronic diseases[28]. Consequently there is increasing interest in searching for novel dietary antioxidants. Various antioxidant assays, such as the superoxide radical scavenging capacity assay[29], oxygen radical absorbance capacity (ORAC) assay[30] and hydroxyl radical scavenging capacity (HOSC) assay[31], were executed in complicated reaction systems and evaluated by spectrophotometric or fluorometric methods. Presence of additional free radical species in these assay systems may lead to overestimation or underestimation of free radical scavenging properties of a tested sample[9]. ESR is a powerful tool to identify the radical purity and concentration consistency, and can be employed for estimating free radical scavenging properties and for studying the interaction between free radicals and antioxidants. A major disadvantage of ESR is that it only works with unpaired electrons. ROS, such as hydrogen peroxide and peroxynitrite, cannot be detected by using ESR.

The screening of antioxidant activities of diet-derived substances is the focus of a large number of articles. Most of those articles are *in vitro* studies, which might not reflect the real function of a food in an organism. Although cell-containing antioxidant activity assay systems are also *in vitro* studies, they can be more representative of the *in vivo* oxidative status and can generate substantial biological information, more valuable than that provided by cell-free systems. A previous study by Panganamala *et al.* indicated that agents which scavenge hydroxyl radicals may inhibit platelet aggregation[32]. In accordance with this, we succeeded in screening C-phycocyanin[33] and alpha-Naphthoflavone[34] as potent platelet inhibitors using a collagen-stimulated platelet ESR system. In this study, we developed a novel AA-stimulated platelet ESR system which may be useful for screening the antioxidant activities of foodstuffs.

In the AA-stimulated platelet ESR system, we demonstrated the generation of hydroxyl radicals in human platelets following exposure to AA. In the presence of DETC, a SOD inhibitor, both superoxide and hydroxyl radicals were identified, which supports our hypothesis that superoxide anions may be the primary species produced by AA-stimulated platelets via 12-LOX. LOXs are non-heme iron proteins which integrate a molecular oxygen into various positions of AA and other polyunsaturated lipids[35]. There are many LOX isoforms such as p12-LOX in platelets, 5-LOX in neutrophils and 1-LOX in soybeans. Incubation of AA with homogenized human platelets produced the lipid peroxide, 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12S-HPETE)[36]. LOXs are activated by lower concentrations, while they are inactivated by higher concentrations of peroxides[37]. This is consistent with our results of the peak value of AA-induced hydroxyl radical signal generation being detected at 10 μM and decreasing at higher concentrations (Figure 3).

AA-metabolized enzymes, such as COX, LOX and reduced NADP (NADPH) oxidase, are regarded as candidates for ROS generation. All of these candidates were tested using the corresponding inhibitors in each pathway as demonstrated in Table 1. Although the two LOX inhibitors used, hinokitiol and NDGA, were able to inhibit AA-induced ROS release, both inhibitors were reported to possess antioxidant activities[38,39]. Therefore, a more specific pathway responsible for this process was explored. We used enzyme antibodies to block specific pathways because AA-metabolized enzymes are present on a variety of cell membranes. As shown in Table 1, the 12-LOX antibody, but not the COX antibody, significantly reduced ROS generation, suggesting a major role of 12-LOX in AA-induced ROS formation in human platelets. The lack of indomethacin and diphenyliodonium inhibition respectively ruled out a major role for COX and NADPH oxidase.

Previously, Egan *et al*. demonstrated the generation of ROS during the metabolism of AA through COX in vesicular gland microsomes[40,41]. They suggested that the ROS was the hydroxyl radical, which may irreversibly inactivate COX[41] and peroxidase[40]. Our results showed that 12-LOX inhibition could not completely abolish AA-induced hydroxyl radical generation (Table 1), suggesting that AA may switch to the COX pathway to produce hydroxyl radicals. 12-LOX inhibition subsequently produced a $g = 2.005$ radical which our previous study suggested to be a carbon-centered radical derived from the self-destruction of prostaglandin H synthase in AA-stimulated platelets[20]. However, we used a higher concentration (1 mM) of AA without 12-LOX inhibition in the previous study and used a lower concentration (10 μM) of AA with 12-LOX inhibition in this study. Results suggested that AA metabolism primarily occurs through the 12-LOX pathway in human platelets.

In conclusion, we clarified the mechanisms of AA-induced free radical generation in human platelets and developed a cell-containing assay system in which two series of free radicals were generated. This novel AA-stimulated human platelet ESR system may prove to be a useful antioxidant activity assay model.

ACKNOWLEDGMENTS

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REFERENCES

1. Halliwell, B., Gutteridge, J. M. and Cross, C. E. 1992. Free radicals, antioxidants, and human disease: Where are we now? J. Lab. Clin. Med. 119: 598-620.
2. Chen, K. C., Liu, W. H. and Chang, L. S. 2010. Suppression of ERK signaling evokes autocrine Fas-mediated death in arachidonic acid-treated human chronic myeloid leukemia K562 cells. J. Cell Physiol. 222: 625-634.
3. Dymkowska, D. and Wojtczak, L. 2009. Arachidonic acid-induced apoptosis in rat hepatoma AS-30D cells is mediated by reactive oxygen species. Acta. Biochim. Pol. 56: 711-715.
4. Liu, W. H. and Chang, L. S. 2009. Arachidonic acid induces Fas and FasL upregulation in human leukemia U937 cells via Ca^{2+}/ROS-mediated suppression of ERK/c-Fos pathway and activation of p38 MAPK/ATF-2 pathway. Toxicol. Lett. 191: 140-148.
5. Chen, K. C. and Chang, L. S. 2009. Arachidonic acid-induced apoptosis of human neuroblastoma SK-N-SH cells is mediated through mitochondrial alteration elicited by ROS and $Ca^{(2+)}$-evoked activation of p38alpha MAPK and JNK1. Toxicology 262: 199-206.
6. Edderkaoui, M., Hong, P., Vaquero, E. C., Lee, J. K., Fischer, L., Friess, H., Buchler, M. W., Lerch, M. M., Pandol, S. J. and Gukovskaya, A. S. 2005. Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. Am. J. Physiol. Gastrointest. Liver Physiol. 289: G1137-G1147.
7. Kim, C. and Dinauer, M. C. 2006. Impaired NADPH oxidase activity in Rac2-deficient murine neutrophils does not result from defective translocation of p47phox and p67phox and can be rescued by exogenous arachidonic acid. J. Leukoc. Biol. 79: 223-234.
8. Shiose, A. and Sumimoto, H. 2000. Arachidonic acid and phosphorylation synergistically induce a conformational change of p47phox to activate the phagocyte NADPH oxidase. J. Biol. Chem. 275: 13793-13801.
9. Yu, L. L. and Cheng, Z. 2008. Application of electron spin resonance (ESR) spectrometry in nutraceutical and food research. Mol. Nutr. Food. Res. 52: 62-78.
10. Rayner, B. S., Duong, T. T., Myers, S. J. and Witting, P. K. 2006. Protective effect of a synthetic anti-oxidant on neuronal cell apoptosis resulting from experimental hypoxia re-oxygenation injury. J. Neurochem. 97: 211-221.
11. Solomon, A., Golubowicz, S., Yablowicz, Z., Bergman, M., Grossman, S., Altman, A., Kerem, Z. and Flaishman, M. A. 2010. EPR studies of O(2)(*-), OH, and (1)O(2) scavenging and prevention of glutathione depletion in fibroblast cells by cyanidin-3-rhamnoglucoside isolated from fig (Ficus carica L.) fruits. J .Agric. Food Chem. 58: 7158-7165.
12. Kagan, V. E., Kuzmenko, A. I., Tyurina, Y. Y., Shvedova, A. A., Matsura, T. and Yalowich, J. C. 2001. Pro-oxidant and antioxidant mechanisms of etoposide in HL-60 cells: role of myeloperoxidase. Cancer Res. 61: 7777-7784.
13. Stapelberg, M., Gellert, N., Swettenham, E., Tomasetti, M., Witting, P. K., Procopio, A. and Neuzil, J. 2005. Alpha-tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: mechanism and the role of oxidative stress. J. Biol. Chem. 280: 25369-25376.
14. Iuliano, L., Pedersen, J. Z., Pratico, D., Rotilio, G. and Violi, F. 1994. Role of hydroxyl radicals in the activation of human platelets. Eur. J. Biochem. 221: 695-704.
15. Caccese, D., Pratico, D., Ghiselli, A., Natoli, S., Pignatelli, P., Sanguigni, V., Iuliano, L. and Violi, F. 2000. Superoxide anion and hydroxyl radical release by collagen-induced platelet aggregation--role of arachidonic acid metabolism. Thromb. Haemost. 83: 485-490.
16. Leo, R., Pratico, D., Iuliano, L., Pulcinelli, F. M., Ghiselli, A., Pignatelli, P., Colavita, A. R., FitzGerald, G. A. and Violi, F. 1997. Platelet activation by superoxide anion and hydroxyl radicals intrinsically generated by platelets that had undergone anoxia and then reoxygenated. Circulation 95: 885-891.
17. Singh, D., Greenwald, J. E., Bianchine, J., Metz, E. N. and Sagone, A. L., Jr. 1981. Evidence for the generation of hydroxyl radical during arachidonic acid metabolism by human platelets. Am. J. Hematol. 11: 233-240.
18. Zuo, L., Christofi, F. L., Wright, V. P., Bao, S. and Clanton, T. L. 2004. Lipoxygenase-dependent superoxide release in skeletal muscle. J. Appl. Physiol. 97: 661-668.
19. Hsiao, G., Lin, K. H., Chang, Y., Chen, T. L., Tzu, N. H., Chou, D. S. and Sheu, J. R. 2005 Protective mechanisms of inosine in platelet activation and cerebral ischemic damage. Arterioscler. Thromb. Vasc. Biol. 25: 1998-2004.
20. Chou, D. S., Hsiao, G., Shen, M. Y., Tsai, Y. J., Chen, T. F. and Sheu, J. R. 2005. ESR spin trapping of a carbon-centered free radical from agonist-stimulated human platelets. Free Radic. Biol. Med. 39: 237-248.
21. Pollock, W. K., Rink, T. J. and Irvine, R. F. 1986. Liberation of [3H]arachidonic acid and changes in cytosolic free calcium in fura-2-loaded human platelets stimulated by ionomycin and collagen. Biochem. J. 235: 869-877.
22. Finkelstein, E., Rosen, G. M., Rauckman, E. J. and Paxton, J. 1979. Spin trapping of superoxide. Mol. Pharmacol. 16: 676-685.
23. Frejaville, C., Karoui, H., Tuccio, B., Le Moigne, F., Culcasi, M., Pietri, S., Lauricella, R. and Tordo, P. 1995. 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide: a new efficient phosphorylated nitrone for the in vitro and in vivo spin trapping of oxygen-centered radicals. J. Med. Chem. 38: 258-265.
24. Vasquez-Vivar, J., Kalyanaraman, B. and Kennedy, M. C. 2000. Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation. J. Biol. Chem. 275: 14064-14069.
25. Vasquez-Vivar, J., Hogg, N., Martasek, P., Karoui, H.,

Tordo, P., Pritchard, K. A. Jr. and Kalyanaraman, B. 1999. Effect of redox-active drugs on superoxide generation from nitric oxide synthases: biological and toxicological implications. Free Radic. Res. 31: 607-617.

26. Suzuki, H., Ueda, T., Juranek, I., Yamamoto, S., Katoh, T., Node, M. and Suzuki, T. 2000. Hinokitiol, a selective inhibitor of the platelet-type isozyme of arachidonate 12-lipoxygenase. Biochem. Biophys. Res. Commun. 275: 885-889.
27. Mikuni, M., Yoshida, M., Hellberg, P., Peterson, C. A., Edwin, S. S., Brannstrom, M. and Peterson, C. M. 1998. The lipoxygenase inhibitor, nordihydroguaiaretic acid, inhibits ovulation and reduces leukotriene and prostaglandin levels in the rat ovary. Biol. Reprod. 58: 1211-1216.
28. Park, Y. K., Park, E., Kim, J. S. and Kang, M. H. 2003. Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. Mutat. Res. 529: 77-86.
29. Wang, S. Y. and Jiao, H. 2000. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J. Agric. Food Chem. 48: 5677-5684.
30. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A. and Prior, R. L. 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J. Agric. Food Chem. 50: 4437-4444.
31. Moore, J., Yin, J. J. and Yu, L. L. 2006. Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation. J. Agric. Food Chem. 54: 617-626.
32. Panganamala, R. V., Sharma, H. M., Heikkila, R. E., Geer, J. C. and Cornwell, D. G. 1976. Role of hydroxyl radical scavengers dimethyl sulfoxide, alcohols and methional in the inhibition of prostaglandin biosynthesis. Prostaglandins 11: 599-607.
33. Hsiao, G., Chou, P. H., Shen, M. Y., Chou, D. S., Lin, C. H. and Sheu, J. R. 2005. C-Phycocyanin, a very potent and novel platelet aggregation inhibitor from *Spirulina platensis*. J. Agric. Food Chem. 53: 7734-7740.
34. Hsiao, G., Chang, C. Y., Shen, M. Y., Chou, D. S., Tzeng, S. H., Chen, T. F. and Sheu, J. R. 2005. alpha-Naphthoflavone, a potent antiplatelet flavonoid, is mediated through inhibition of phospholipase C activity and stimulation of cyclic GMP formation. J. Agric. Food Chem. 53: 5179-5186.
35. Hodnick, W. F., Kung, F. S., Roettger, W. J., Bohmont, C. W. and Pardini, R. S. 1986. Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids. A structure-activity study. Biochem. Pharmacol. 35: 2345-2357.
36. Hamberg, M. and Samuelsson, B. 1974. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. Proc. Natl. Acad. Sci. U.S.A. 71: 3400-3404.
37. Kulkarni, A. P. 2001. Lipoxygenase--a versatile biocatalyst for biotransformation of endobiotics and xenobiotics. Cell Mol. Life Sci. 58: 1805-1825.
38. Diouf, P. N., Delbarre, N., Perrin, D., Gerardin, P., Rapin, C., Jacquot, J. P. and Gelhaye, E. 2002. Influence of tropolone on *Poria placenta* wood degradation. Appl. Environ. Microbiol. 68: 4377-4382.
39. Van Wauwe, J. and Goossens, J. 1983. Effects of antioxidants on cyclooxygenase and lipoxygenase activities in intact human platelets: comparison with indomethacin and ETYA. Prostaglandins 26: 725-730.
40. Egan, R. W., Gale, P. H. and Kuehl, F. A., Jr. 1979. Reduction of hydroperoxides in the prostaglandin biosynthetic pathway by a microsomal peroxidase. J. Biol. Chem. 254: 3295-3302.
41. Egan, R. W., Paxton, J. and Kuehl, F. A., Jr. 1976. Mechanism for irreversible self-deactivation of prostaglandin synthetase. J. Biol. Chem. 251: 7329-7335.