Research Article

Fatty acids in tea shoots (*Camellia sinensis* (L.) O. Kuntze) and their effects on the growth of retinal RF/6A endothelial cell lines

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Chemo-protective effects of tea on ocular diseases were recorded in Chinese pharmacopoeia about 2000 years ago by eating tea. In the present study, contents of fatty acids (FAs) in tea shoots were determined by capillary GC; and the growth of RF/6A cells was also investigated by exposure to various representative FAs existing in tea shoots with pathologically relevant concentrations (40 – 500 μM) by ameliorated MTT assay and flow cytometry. Electron spin resonance (ESR) was used to measure oxygen consumption and investigate the free radical scavenging ability of linoleic acid (LA). Results showed that the most abundant long chain FAs were palmitic, linoleic, and α-linolenic acid in tea shoots; some RF/6A cells became suspended in culture medium treated by a high dose of both saturated and unsaturated FAs, but no apoptosis was observed. Moreover, it seemed that those FAs with different structure had various effects on the cell proliferation at their relatively low concentrations, LA expressed antioxidant activity in this study, which might be an important mechanism on the protection of eyes.

Keywords: ESR / Long chain fatty acids / RF/6A Cells / Retinal / Tea

Received: August 15, 2006; revised: October 14, 2006; accepted: October 16, 2006

1 Introduction

It is well known that tea is a health beverage and has excellent chemo-protective effects on many diseases, such as cancer, inflammation, and so on [1 – 3]. Tea was used as a medicine in China about 2000 years ago, and its preventive effects from ocular diseases were recorded at that time by eating tea [4]. So far, the main components in tea and mechanisms on the protection of eyes have not been clear yet, which might become a compelling topic in the near future. Recently, a few reports revealed that catechins and fatty acids (FAs) that existed in tea were relative to preventive and therapy effects from some ocular disease [5 – 7]. Especially, FAs were important functional components for developmental and pathological changes of eyes, and would exert pivotal effects on the protection of eyes depending on the functional composition of tea. FAs in tea shoots were considered to have a relationship with faculty of sight in Chinese traditional medicine.

Progress in lipid research has highlighted several pathways through which free FAs were able to cause cellular and functional alterations. These included changes in membrane composition and function, and in the regulation of gene expression and protein modification [8, 9]. Such pathways were likely to be relevant to endothelial cells. Previously, different FAs have been shown to have respective effects on the cell proliferation.

In the present paper, we first investigated the FA composition of tea shoots and their effects on the growth of retinal RF/6A endothelial cell lines. The oxygen consumption of linoleic acid (LA) and its radical scavenging ability were also studied in order to reveal the mechanism of this FAPUFA in preventing from ocular disease and protecting eyes.

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Abbreviations: ESR, electron spin resonance; **FAs,** fatty acids; **LA,** linoleic acid

2 Materials and methods

2.1 Materials

Purified preparation of FAs (palmitic acid, LA, and α-linolenic acid) and D-α-Phosphatidylcholine, Dipalmitoyl (DPPC), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma (St. Louis, MO, USA). The rhesus macaque choroids-retinal endothelial cell (RF/6A) was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. DMEM was purchased from GIBCO (Grand Island, NY, USA). ANNEXIN V kit was a product of Caltag Laboratories (USA). 4-Oxo-2,2,6,6-tetramethylpiperidine-d16-1-15N-oxyl (N-PDT) was from Cambridge Isotope Laboratories (Andover, MA, USA). 1-Stearoyl-2-octadec-cis9,cis12-dienoyl-*sn*-glycero-3-phosphorylcholine [PC (LA)] was purchased from Matreya (Pleasant Gap, PA, USA). Egg yolk phosphatidylcholine (Egg PC) was obtained from Avanti Polar Lipids (Alabaster, AL, USA) and 2,2′-azobis(2,4-dimethylvaleronitrile (AMVN) was acquired from Polysciences (Warrington, PA, USA). All other chemicals were of extra-pure grade or analytical grade.

2.2 FAs analysis of tea shoots

Standard tea shoots, comprising an apical bud and the three terminals of clone Longjing 43, were harvested from a tea garden of Zhejiang University (P. R. China). Total lipids were extracted from 2 g of bud, first, second, and third leaf individually with 30 mL of $CHCl_3$–MeOH (2:1) for 1 h. The bulk extract was evaporated to dryness in a rotary vacuum evaporator at 45°C and then saponified in H_2SO_4–MeOH (2:230) at 85°C for 1 h. The corresponding methyl esters of FAs were analyzed by capillary GC with FID using a DEGS column, programmed from 160 to 220°C at 5°C/min(GC-14B, Shimadzu, Japan).

2.3 FAs solution preparations

Sufficient FAs were dissolved in preheated 0.1 N NaOH and diluted 1:10 in prewarmed (50°C) DMEM containing 1% w/v BSA, to give a final FA concentration of 2.0 mM. Stock FA solutions were filter-sterilized and diluted with cell culture media to use in the study. Control carrier was a medium that contained 0.1 N NaOH and BSA but no FAs. The pH of preparations was adjusted to 7.4 when required.

2.4 Cell culture

RF/6A cells were cultured in DMEM supplemented with 10% fetal bovine serum v/v and 100 U/mL penicillin and 100 U/mL streptomycin in an atmosphere of 5% CO_2 at 37°C (Shellab, USA). The cells were maintained with a medium change every 24–48 h, before being used in experiments.

2.5 Proliferation and ratio of cells adhering on plate detected by ameliorated MTT assay

Cells (1×10^5) were seeded in a 96-well plate for 24 h, the medium was replaced by 1% BSA–DMEM containing 40, 80, 120, 160, 200, 300, 400, or 500 μM palmitic, linoleic, α-linolenic acid, respectively. Control cells were exposed to a growth medium supplemented control carrier. Cells were incubated for 0–48 h in the test medium, and culture medium was dropped (floating cells were dropped). 3-[4, 5-Dimethythiazol-2-yl]-2,5-Diphenyltetrazolium bromide (MTT) (20 μL) at the concentration of 5 mg/mL-PBS was added to each well, and then 150 μL of DMSO was added after incubation for 4 h. Absorbance was measured for each well on a Microplate Reader (Thermal Lab system, Finland). Ratio of cells adhering on plate was calculated as follows:

$$\text{Ratio of cells adhering on}(\%) = \frac{\text{ABS}_{(\text{treatment,with cells})} - \text{ABS}_{(\text{tretatment,without cells})}}{\text{ABS}_{(\text{control,with cells})}} \times 100$$

2.6 Gel electrophoresis and DNA fragmentation analysis

Cells (1×10^5) were seeded onto 25 cm^2 plates for 24 h and incubated with different concentrations of FAs for 24 and 48 h, respectively. Following this treatment, the cells were collected and centrifuged at 4°C for 5 min (1500 rpm). After an additional wash with PBS, the cells were lysed in 400 μL of cytolysis solution (0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, pH 8.0, 0.5% SDS) and proteinase (50 μg/mL) at 50°C overnight. Then 400 μL of chloroform/isoamylalcohol (24:1) was added and mixed well. The cells were centrifuged at 4°C for 10 min (12 000 rpm) and the supernatant was transferred to a new tube; two volume of ice-cold ethanol was added and kept on ice for 5 min. Then the cells were again centrifuged at 4°C for 10 min (12 000 rpm), the pellets were washed with 70% ethanol twice. After ethanol was entirely volatilized, DNA was dissolved in 30 μL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA samples were loaded onto a 1.2% w/v agarose gel (containing 0.5 μg/mL of ethidium bromide) and the DNA fragments were separated by electrophoresis (Amersham Biosciences) at 5 V/cm for 1 h in TAE buffer. The DNA was visualized using an imaging system (Shanghai Tianneng, P. R. China) and photographed using an Alpha-imager 2000 digital camera.

2.7 Flow cytometry and cell damage assay

Cells were reseeded at a density of 2×10^5 cells/cm^2 onto 50 cm^2 plates for 48 h and were treated with different concentrations of FA for 24 h. Floating and trypsinized adherent cells were collected by centrifugation (1500 rpm,

10 min). Cells were stained with biotin-conjugated Annexin V, FITC-conjugated streptavidin, and PI using the Annexin V-Biotin Apoptotic Detection kit according to the manufacturer's protocol. Apoptotic cells were subsequently counted and analyzed by flow cytometry (FACS sort, BD, USA).

2.8 Analysis of LA on scavenging DPPH free radical and measurement of oxygen consumption by Electron spin resonance (ESR)

The DPPH stable radical scavenging activity of LA in test samples was estimated by ESR according to the method of Yamaguchi *et al*. [10]. Reaction mixture contained 50 μL of test sample with or without 5 mol% of LA and 0.25 mM DPPH in DPPC liposome or in DPPC ethanol solution. Samples were incubated at 60°C for 45 min. All measurements were performed at 60°C. In this work, the spin label 4-oxo-2,2,6,6-tetramethylpiperidine-d16-1-15N-oxyl (^{15}N-PDT) was used, Egg PC liposome (with or without 5 mol% LA) containing 10 mM AMVN and 0.2 mM ^{15}N-PDT was incubated at 45°C for 30 min then transferred to a 50 μL capillary. ESR spectra were recorded at 2 min intervals for 15 min. All ESR spectra were recorded at the low field line of N-PDT and at 37°C.

Conventional ESR spectra were obtained with a Varian E-109 X-band spectrometer with a variable temperature controller accessory. ESR signals were recorded with 10 mw (for DPPH study) and 0.5 mw (for ESR oximetry) incident microwave power and 100 kHz field modulations of 2 G (for DPPH study) and 0.05 G (for ESR oximetry). The field scan range was 100 G (for DPPH study) and 1 G (for ESR oximetry).

3 Results

3.1 FAs analysis of tea shoots

The variation of FA contents in different tea shoots was presented in Table 1. It was clear that contents of FAs increased from bud to third leaf and was the least in the stem of tea shoots. The gas chromatogram of FAs in the third tea leaf is shown in Fig. 1. The most abundant PUFAs in tea shoots were linoleic and α-linolenic acid, and it showed that the main saturated FA in tea shoots was palmitic acid.

3.2 Proliferation and ratio of RF/6A cells adhering on plate

Adhesive character of retinal cells was an important factor affecting the occurence and progression of many ocular diseases, and ratio of cells adhering on plate was detected by ameliorated MTT assay in this work. As shown in Fig. 2, there was no significant difference between the proliferation of RF/6A cells cultured with the palmitic acid below the concentration of 400 μM and the control cells cultured without FAs both for 12–48 h. However, it was shown that 500 μM palmitic acid significantly inhibited ratio of RF/6A cells adhering on plate ($p < 0.01$).

When treated with a moderate concentration (<200 μM) of LA for 12 and 24 h, the cell proliferation was slightly stimulated compared with the control ($p < 0.05$ at 80, 120, and 160 μM). Although there was no significant difference between the LA supplemented media (40–200 μM) and the control at the time of 36 h, proliferation of RF/6A cells was increased at 48 h ($p < 0.05$ at 80, 120, and 160 μM). On the other hand, ratio of RF/6A cells adhering on plate was significantly inhibited within 12–48 h when treated with a higher dose of LA ($p < 0.01$ at 400 and 500 μM). These results were consistent with the results of microscopic observation, which showed that a number of cells were suspended in the medium when treated with such a high concentration (Fig. 3B).

The addition of 40–200 μM α-linolenic acid did not have distinct effects on cell proliferation at the time of 12, 36, and 48 h, but proliferation of RF/6A cells was significantly increased when treated for 24 h ($p < 0.05$ at 40, 80, 160 μM, $p < 0.01$ at 120 μM). When the concentration of α-linolenic acid was more than 300 μM, the ratio of RF/6A cells adhering on plate was sharply decreased. A number of suspended cells were observed in the medium treated with high dose of α-linolenic acid (Fig. 3C). After being treated

Table 1. FA composition of tea *C. sinensis* (L.) O. Kuntze

	FA composition[a] (% peak area)								Total lipids (mg/g fresh wt.)[b]
	Lauric C12	Palmistic C16	Palmitoleic C16:1	Myristic C14	Stearic C18	Oleic C18:1	Linoleic C18:2	α-Linolenic C18:3	
Bud	ND	32.73 ± 1.50	ND	ND	ND	1.78 ± 0.04	35.61 ± 0.83	29.88 ± 0.69	0.72
First leaf	ND	30.01 ± 0.51	ND	ND	1.24 ± 0.51	6.99 ± 0.55	26.12 ± 0.46	35.64 ± 0.73	1.60
Second leaf	ND	29.99 ± 0.97	4.21 ± 0.11	ND	1.35 ± 0.18	9.21 ± 0.38	25.85 ± 0.43	39.38 ± 0.46	4.83
Third leaf	0.56 ± 0.01	26.87 ± 1.27	4.97 ± 0.16	0.01 ± 0.001	2.07 ± 0.34	8.82 ± 0.34	24.33 ± 0.17	31.96 ± 0.30	5.24
Stem	ND	38.98 ± 0.97	3.94 ± 0.99	ND	6.62 ± 0.49	4.44 ± 0.31	24.38 ± 0.52	24.60 ± 0.50	2.26

a) Data were expressed as mean ± SD ($n = 4$); ND: not detected.
b) Total lipids were analyzed by comparing with those FAs of standard.

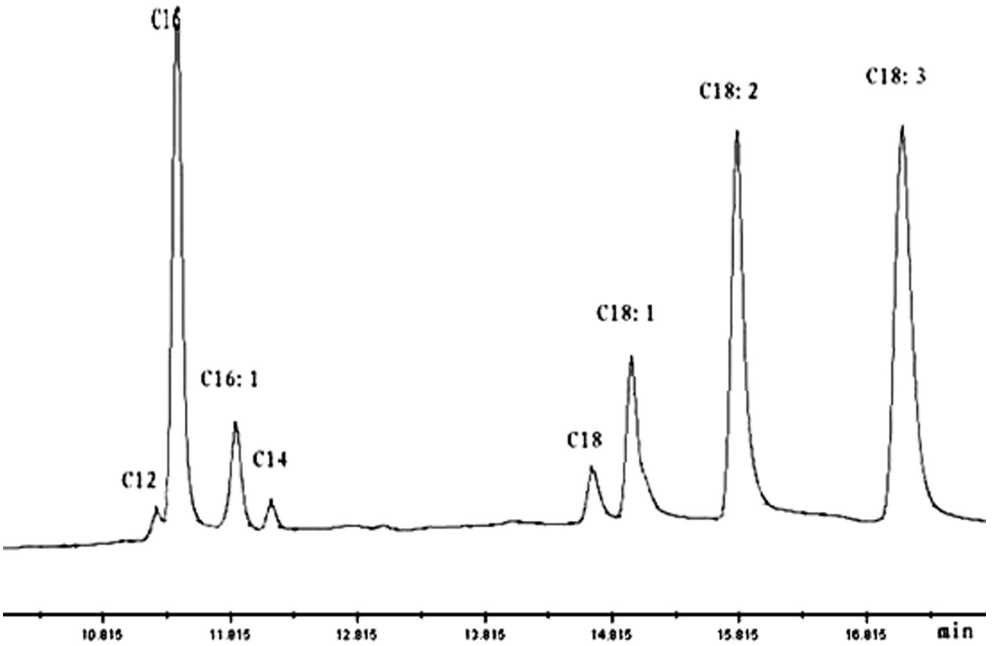

Figure 1. Gas chromatogram of FAME of the third leaf of tea C*amellia sinensis* (L.) O. Kuntze. Identification of FAME was accomplished by comparing the retention times of FAME with that of standard.

with relatively high concentrations of different FAs, the images of RF/6A cells were also taken. From Fig. 3, we found that 500 μM palmitic acid did not affect the morphologic profile of RF/6A cells while 400 μM LA and 300 μM α-linolenic acid reduced the number of adherent cells, respectively.

3.3 Apoptosis analysis

From the above results, the ratio of RF/6A cells adhering on plate was changed with incubation in different concentrations of FAs. How about those suspending cells, alive, dead or apoptosis? We chose different concentrations of FAs depending on the results of the MTT assay (Fig. 2) to investigate the DNA fragmentation of RF/6A cells and flow cytometric analysis. Because the fraction of RF/6A cells adhering on the plate began to decrease significantly when the concentrations of palmitic acid, LA, and α-linolenic acid reached 500, 400, and 300 μM, respectively, we thought these concentrations at that point were appropriate for apoptosis analysis. As shown in Fig. 4, there was no any DNA ladder in the treated cells by agarose gel electrophoresis. In the results of flow cytometric analysis (Table 2), it was clear that palmitic acid did not induce progressive apoptosis and there was no differences in its effects on RF/6A cells compared with the control cells. Linoleic and α-linolenic acid induced slight necrosis at a concentration of 400 and 300 μM within 24 h, the necrosis percentage was 11.93 and 7.00%, respectively (Table 2). The percentage of living cell population was quantified as 95.5, 87.2, and 91.9% in palmitic, linoleic, and α-linolenic acid-treated media, respectively, which indicated that the main cells were alive compared with the control (95.4%). The apoptotic cells were almost negligible in these FAs supplemented media compared to that with BSA only.

Table 2. Percentage of RF/6A cells stained with Annexin V and PI treated with different FAs

%	CK[a)]	Palmitic C16:0 500 μM	Linoleic C18:2 400 μM	α-Linolenic C18:3 300 μM
Necrotic cells	3.52	4.41	11.93	7.00
Apoptotic cells	1.08	0.09	0.87	1.08
Alive cells	95.4	95.5	87.2	91.9

a) treated with control medium containing equivalent amount of BSA compared with 500 μM palmitic acid.

3.4 Free radical scavenging ability of FAs and oxygen consumption analysis by ESR

Redox state was relative to eyes health and pathological changes. Modulating redox state might be an important mechanism of some FAs of tea such as LA (the most excellent antioxidant among palmitic acid, LA and α-linolenic acid) on their preventive effects from ocular diseases. So, we investigated free radical scavenging ability and effects on oxygen consumption rate of LA in lipid system. Figure 5 shows that LA reacted with stable radical DPPH to rapidly quench its ESR signal both in DPPC liposome and DPPC

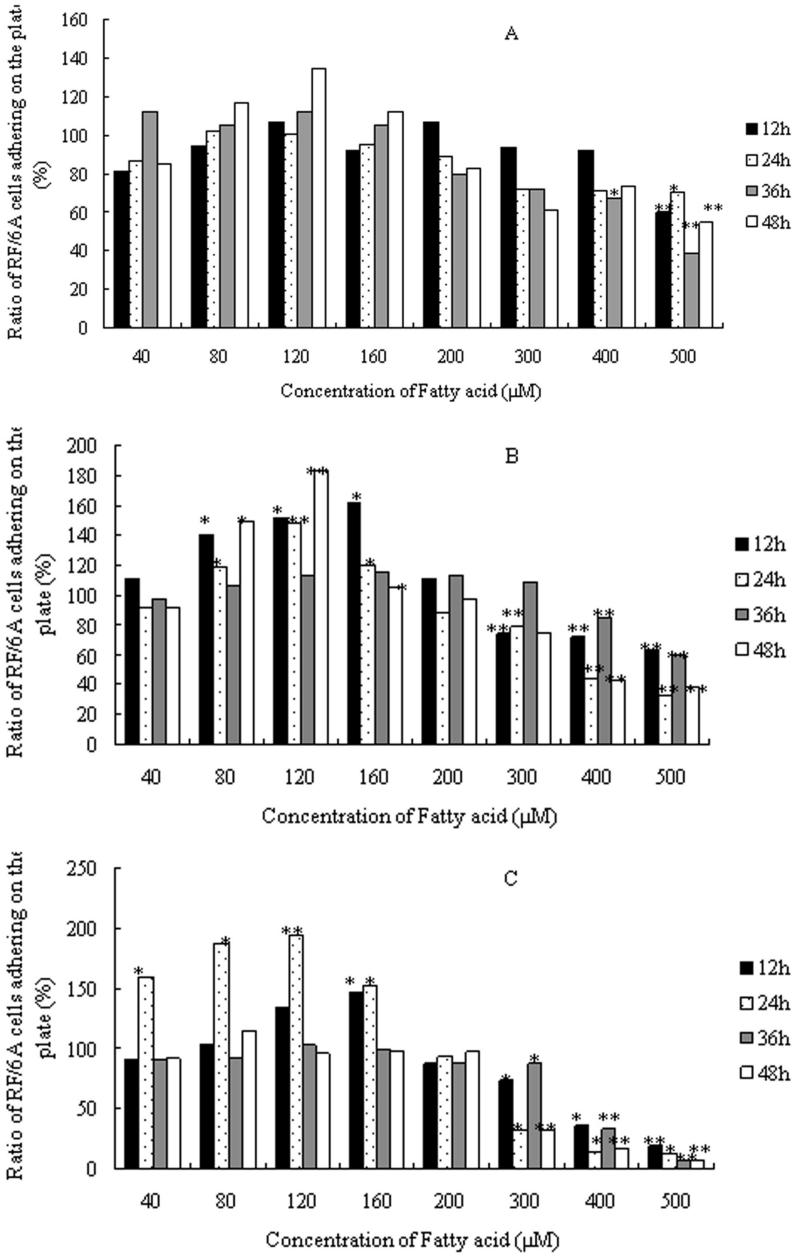

Figure 2. Proliferation and ratio of RF/6A cells adhering on the plate after 12–48 h treatement with different FAs. (A) Treatment with different concentrations of palmitic acid; (B) treatment with different concentrations of LA; (C) treatment with different concentrations of α-linolenic acid. Equivalent amounts of BSA alone were added to control plates. $^{*}p < 0.05$ compared to control, $^{**}p < 0.01$ compared to control.

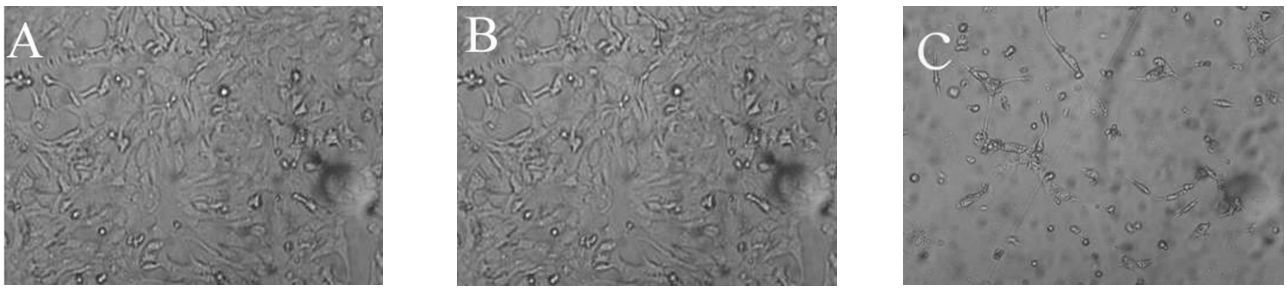

Figure 3. Microscopic images of RF/6A cells treated with a relatively high concentration of varies FAs for 24 h. (A) Adhesive cells after treatment with 500 μM palmitic acid; (B) adhesive and floating cells after treatment with 400 μM LA; (C) adhesive and floating cells after treatment with 300 μM α-linolenic acid.

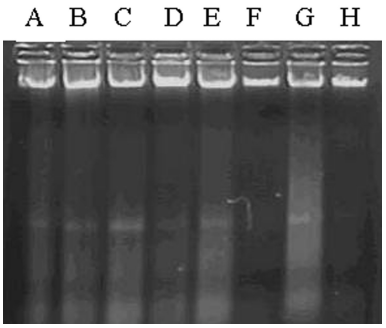

Figure 4. DNA images of RF/6A cells treated with different concentrations of FAs. (A) Treatment with control medium for 24 h; (B) treatment with control medium for 48 h; (C) treatment with 500 μM palmitic acid for 24 h; (D) treatment with 500 μM palmitic acid for 48 h; (E) treatment with 400 μM lineleic acid for 24 h; (F) treatment with 400 μM lineleic acid for 48 h; (G) treatment with 300 μM α-linolenic acid for 24 h; (H) treatment with 300 μM α-linolenic acid for 48 h.

ethanol solution. The intensity of the ESR signals was proportional to the concentration of DPPH radicals, with a stronger signal associated with higher DPPH concentration. Incorporation of 5 mol% synthetic PC containing LA isomers into test systems reduced the ESR signal intensity both in DPPC ethanol solutions and DPPC aqueous liposome suspensions (Figs. 5A and B), which suggested that LA could scavenge DPPH radical in the above two systems under the experimental conditions.

Oxygen consumption rate was estimated by ESR method in egg PC liposome (with or without LA) system. Figure 6 illustrates the repeated scans of the ESR signal of spin label during lipid oxidation. The slower changes of the line width in the repeated scans suggested that the oxygen consumption rate in Egg PC containing 5 mol% LA system was lower than that of Egg PC alone.

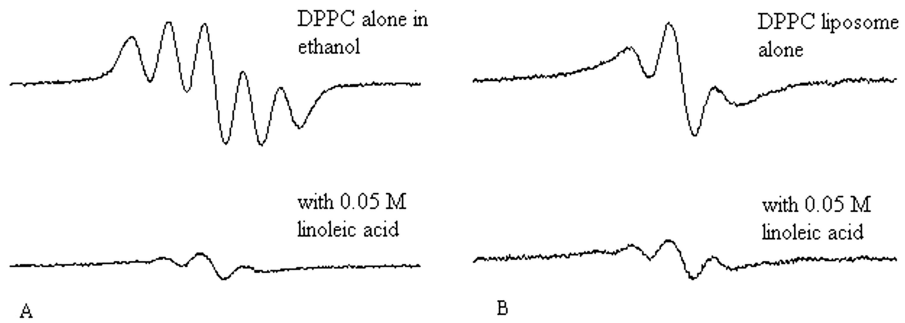

Figure 5. DPPH radical-LA reaction in DPPC testing system. ESR signal was recorded with 10 mW incident microwave and 100 kHz field modulation of 2 G at 60°C. Reaction mixture containing 50 μL of test sample with or without 5 mol% of LA and 0.25 mM DPPH in DPPC liposome or in DPPC ethanol solution. Samples were incubated at 60°C for 45 min. All measurements were performed at 60°C. The ESR signal of DPPH radical was measured in ethanol solution (A) and in liposomes (B). Pure DPPC and 5 mol% mixtures of LA in DPPC were measured.

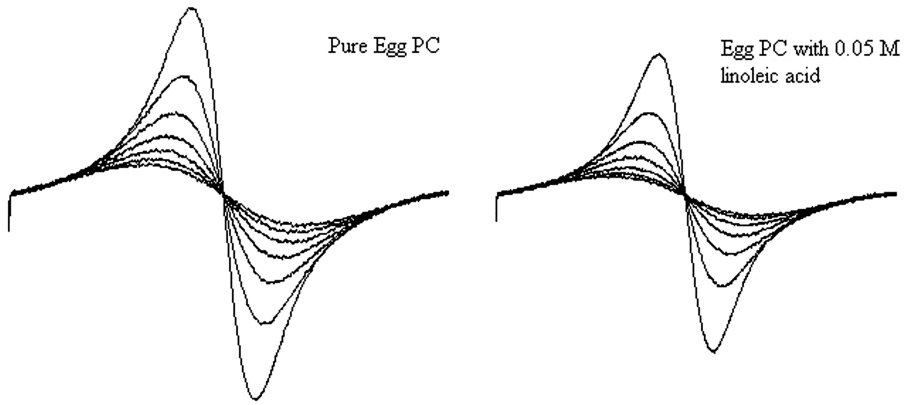

Figure 6. Time dependence of ESR signal during lipid peroxidation in pure Egg PC and Egg PC mixed with 5 mol% of LA liposomes. The low field line of 15N-PDT spin label ESR spectra was recorded every 2 min. The ESR signal was recorded with 0.5 mW incident microwave and 100 kHz field modulation of 0.05 G at 37°C. Lipid peroxidation was initiated by using AMVN.

4 Discussion

FAs were important components in tea shoots because of its health function and its effects on character of tea [11]. Metabolism of FAs in tea shoots was emphasized in the past reports, and its bioactivity to health was ignored. With the progress of protective effects of tea on many diseases, FAs of tea were explored as kinds of heath compositions. Till now, few reports about the compositions and the contents of FAs in tea shoots have been made. In this paper, we investigated the composition and the contents of FAs in different parts of tea shoots in order to reveal its relations to protective function of tea on ocular diseases, and the results showed that linoleic, α-linolenic acid, and palmitic acid were the main kinds of FAs in tea shoots. It might be a feasible way to clarify the health function of tea on eyes by investigating their effects on retinal cells.

FA was focused on by ophthalmologist because of its closed relations with development of vision, especially some long chain FAs including DHA, DPA, *etc*. [12 – 16]. As precursors of DHA/DPA, LA, and α-linolenic acid would affect the development of vision and pathological changes of eyes [17]. Udell *et al.* [18] found that alpha-linolenic acid supplemented diets improved the DHA status of infants. Lots of studies confirmed the favorable protective effects of alpha-linolenic acid on eyes [19 – 21]. As an excellent antioxidant, regulation of redox state might be an important aspect of LA in its preventive effects from ocular diseases, which was proved by our ESR results. Past reports showed that excessive palmitic acid would accelerate retinal pathological changes induced by diabetes [22]. But, effects of palmitic acid on other ocular diseases maintained unknown. Interestingly, few studies about effects of the three FAs on growth of retinal cells have been made till now. In this work, we investigated the effects of three FAs on coherent activity, apoptosis, and growth of RF/6A cells. Three FAs did not induce apoptosis of RF/6A cells, and they decreased the ratio of RF/6A cells adhering on plate in certain conditions. Effects of LA and α-linolenic acid on coherent activity and growth of RF/6A cells were more effective than palmitic acid. LA and α-linolenic acid were main components of tea in protections of eyes. Moreover, their protective mechanisms need to be investigated further.

Expression of adhesion molecule in retinal cells was relative to many ocular diseases, especially diabetic retinopathy [23, 24], and distributed on endothelial cells and leukocytes, and participated in the recruitment of leukocytes to sites of tissue injury and inflammation. Previously, some studies about inhibitory expression of some kinds of adhesion molecule of long chain FAs were reported [25]. Effects of palmitic acid, LA and α-linolenic acid on expression of adhesion molecule in retinal cells have not been investigated so far. In the present paper, we evaluated effects of the three FAs on expression of adhesion molecule in RE/6A cells for the first time. The three FAs could inhibit an expression of adhesion molecule in RF/6A cells, and LA and α-linolenic acid were more sensitive than palmitic acids. Regulating the expression of adhesion molecule, inhibiting inflammation and exerting protective effects on some ocular diseases such as diabetic retinopathy were important pathways for the protection of eyes by tea consumption.

In conclusion, LA, α-linolenic acid, and palmitic acid were the main components in tea shoots, and they regulated retinal pathological changes by affecting growth of retinal cells, regulating expression of adhesion molecule, and modulating the redox state of retinal cells. Effects of the three FAs were different because of their different structures. Hence, it might be an interesting topic to investigate the interactions and ratios of concentrations among FAs existing in tea shoots and their effect on the protection of eyes.

5 References

[1] Srinivasan, P., Sabitha, K. E., Shyamaladevi, C. S., *Chem. Biol. Interact.* 2006, *162*, 149 – 156.

[2] Kuzuhara, T., Sei, Y., Yamaguchi, K., Suganuma, M., Fujiki, H., *J. Biol. Chem.* 2006, *281*, 17446 – 17456.

[3] Widlansky, M. E., Duffy, S. J., Hamburg, N. M., Gokce, N., *et al.*, *Free Radic. Biol. Med.* 2005, *38*, 499 – 506.

[4] Chuan, C., *Pharmacology of Tea*, Chinese Prospect Press, Beijing 1987, in Chinese.

[5] Gao, Y. Y., Di Pascuale, M. A., Li, W., Baradaran-Rafii, A., *et al.*, *Br. J. Ophthalmol.* 2005, *89*, 1468 – 1473.

[6] Blanco, A. R., Sudano-Roccaro, A., Spoto, G. C., Nostro, A., Rusciano, D., *Antimicrob. Agents Chemother.* 2005, *49*, 4339 – 4343.

[7] Vinson, J. A., Zhang, J., *J. Agric. Food Chem.* 2005, *53*, 3710 – 3713.

[8] Sivalingam, A., Kenney, J., Brown, G. C., Benson, W. E., Donoso, L., *Arch. Ophthalmol.* 1990, *108*, 869 – 872.

[9] Kon, C. H., Occleston, N. L., Aylward, G. W., Khaw, P. T., *Invest. Ophthalmol. Vis. Sci.* 1999, *40*, 705 – 712.

[10] Yamaguchi, F., Ariga, T., Yoshimura, Y., Nakazawa, H., *J. Agric. Food Chem.* 2000, *48*, 180 – 185.

[11] Ryoyasu, S., Tadakazu, T., *Plant Cell Physiol.* 1972, *13*, 991 – 998.

[12] Jensen, C. L., Voigt, R. G., Prager, T. C., Zou, Y. L., *et al.*, *Am. J. Clin. Nutr.* 2005, *82*, 125 – 132.

[13] Berson, E. L., Rosner, B., Sandberg, M. A., Weigel-DiFranco, C., *et al.*, *Arch. Ophthalmol.* 2004, *122*, 1306 – 1314.

[14] Lauritzen, L., Jorgensen, M. H., Mikkelsen, T. B., Skovgaard, M., *et al.*, *Lipids.* 2004, *39*, 195 – 206.

[15] McBrien, N. A., Norton, T. T., *Exp. Eye Res.* 1994, *59*, 475 – 486.

[16] Zhou, D., Zaiger, G., Ghebremeskel, K., Crawford, M. A., Reifen, R., *Prostaglandins Leukot. Essent. Fatty Acids* 2004, *71*, 383 – 389.

[17] Carnielli, V. P., Wattimena, D. J., Luijendijk, I. H., Boerlage, A., *et al.*, *Pediatr. Res.* 1996, *40*, 169 – 174.

[18] Udell, T., Gibson, R. A., Makrides, M., *Lipids* 2005, *40*, 1 – 11.

[19] Okuyama, H., Ohishi, M., Fukuma, A., Sato, Y., *et al.*, *World Rev. Nutr. Diet.* 2001, *88*, 35 – 40.

[20] Su, H. M., Bernardo, L., Mirmiran, M., Ma, X. H., *et al.*, *Pediatr. Res.* 1999, *45*, 87 – 93.

[21] Delton-Vandenbroucke, I., Grammas, P., Anderson, R. E., *J. Lipid Res.* 1997, *38*, 147 – 159.

[22] Cacicedo, J. M., Benjachareowong, S., Chou, E., Ruderman, N. B., Ido, Y., *Diabetes* 2005, *54*, 1838 – 1845.

[23] Aronni, S., Cortes, M., Sacchetti, M., Lambiase, A., *et al.*, E*ur. J. Ophthalmol.* 2006, *16*, 17 – 23.

[24] Soedamah-Muthu, S. S., Chaturvedi, N., Schalkwijk, C. G., Stehouwer, C. D., *et al.*, *J. Diabetes Complicat.* 2006, *20*, 188 – 195.

[25] Ferrante, A., Robinson, B. S., Singh, H., Jersmann, H. P., *et al.*, *Circ. Res.* 2006, *99*, 34 – 41.

